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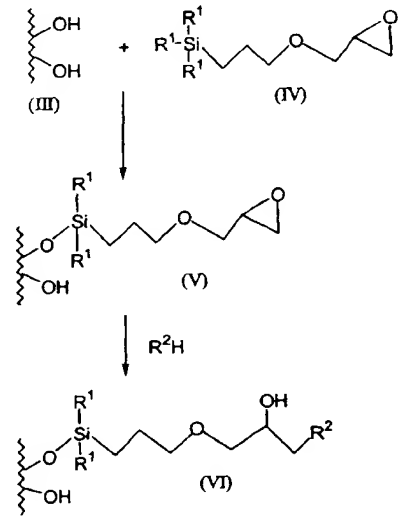
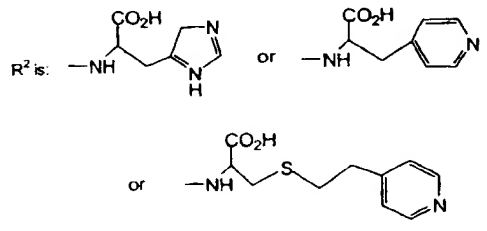
(54) Title: pH DEPENDENT ION EXCHANGE MATRIX AND METHOD OF USE IN THE ISOLATION OF NUCLEIC ACIDS

(57) Abstract

pH dependent ion exchange matrices are provided, with methods for making such matrices, and methods for using such matrices to isolate a target nucleic acid, as such as plasmid DNA, chromosomal DNA, or RNA from contaminants, including proteins, lipids, cellular debris, or other nucleic acids. Each pH dependent ion exchange matrix of this invention comprises at least two different ion exchange functional groups, one of which is capable of acting as an anion exchanger at a first pH, and the other of which is capable of acting as a cation exchanger at a second, higher pH. The matrix has an overall neutral charge in a pH range between the first and second pH. The pH dependent ion exchange matrices of the present invention are designed to bind to the target nucleic acid at a pH wherein the overall charge of the matrix is positive, and to release the target nucleic acid as the pH of the surrounding solution is increased. The target nucleic acid can be released from the pH dependent matrix in little or no salt and at about a neutral pH. The matrices and methods of this invention enable one to isolate a target nucleic acid in very few steps, without the use of hazardous chemicals. Target nucleic acids isolated using the pH dependent ion exchange matrices according to the present invention can be used immediately without further extraction or isolation.



wherein, R^1 is -OH, $-OCH_3$, or $-OCH_2CH_3$; and



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pH DEPENDENT ION EXCHANGE MATRIX AND METHOD OF USE IN THE ISOLATION OF NUCLEIC ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Patent Application Serial No. 09/312,172, filed 14 May 1999.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

TECHNICAL FIELD

This invention relates generally to materials and methods for isolating a target nucleic acid, such as plasmid DNA, chromosomal DNA, total RNA, mRNA, or RNA/DNA hybrids from contaminants, such as proteins, lipids, cellular debris, and non-target nucleic acids. This invention relates, particularly, to pH dependent ion exchange matrices with the capacity to adsorb a target nucleic acid in the presence of a solution at a first pH and to desorb the target nucleic acid in the presence of a second solution at a second pH which is different from the first pH. This invention also relates to methods of making and using such pH dependent ion exchange matrices in isolating target nucleic acids.

BACKGROUND OF THE INVENTION

Many molecular biological techniques such as reverse transcription, cloning, restriction analysis, amplification and sequencing require that nucleic acids used in the techniques be substantially free of contaminants capable of interfering with such processing or analysis procedures. Such contaminants generally include substances that block or inhibit chemical reactions, (e.g. substances that block or inhibit nucleic acid hybridizations, enzymatically catalyzed reactions and other types of reactions used in molecular biological techniques), substances that catalyze the degradation or depolymerization of a nucleic acid or other biological material of interest, or substances which block or mask detection of the nucleic acid of interest. Substances of this last type can block or mask by providing a "background" indicative of the presence in a sample of a quantity of a nucleic acid of interest, (also referred to herein as a "target nucleic acid") when the nucleic acid of interest is not, in fact, present in the sample. Contaminants also include macromolecular

substances from the *in vivo* or *in vitro* medium from which a target nucleic acid is isolated, macromolecular substances such as enzymes, other types of proteins, polysaccharides, or polynucleotides, as well as lower molecular weight substances, such as lipids, low molecular weight enzyme inhibitors, oligonucleotides, or non-target nucleic acids. Contaminants can also be introduced into a target biological material from chemicals or other materials used to isolate the material from other substances. Common contaminants of this last type include trace metals, dyes, and organic solvents.

Obtaining target nucleic acid sufficiently free of contaminants for molecular biological applications is complicated by the complex systems in which the target nucleic acid is typically found. These systems, e.g., cells from tissues, cells from body fluids such as blood, lymph, milk, urine, feces, semen, or the like, cells in culture, agarose or polyacrylamide gels, or solutions in which target nucleic acid amplification has been carried out, typically include significant quantities of contaminants from which the target nucleic acid of interest must be isolated before being used in a molecular biological procedure.

The earliest techniques developed for use in isolating target nucleic acids from such complex systems typically involve multiple organic extraction and precipitation steps. Hazardous chemicals, such as chloroform and phenol or mixtures thereof, were used in most such procedures. Closed circular nucleic acid molecules, such as plasmid DNA, was typically isolated further by ultra-centrifugation of plasmid DNA in the presence of cesium chloride and ethidium bromide. See, e.g., Molecular Cloning, ed. by Sambrook et al. (1989), pp. 1.42-1.50. Ethidium bromide is a neurotoxin. Removal of both ethidium bromide and cesium chloride from the resulting band of plasmid DNA obtained by ultracentrifugation was required before the DNA could be used in downstream processing techniques, such as sequencing, transfection, restriction analysis, or the polymerase chain reaction.

In recent years, many different matrices have been developed for use in the isolation of nucleic acids from complex biological materials. For example, matrices have been developed for the isolation of nucleic acids by ion-exchange chromatography (e.g., *J. of Chromatog.* 508:61-73 (1990); *Nucl. Acids Research* 21(12):2913-2915 (1993); U.S. Pat. No.'s 5,856,192; 5,82.988; 5,660,984; and 4,699,717), by reversed phase (e.g. Hirbayashi et al., *J. of Chromatog.* 722:135-142 (1996); U.S. Pat. No's 5,057,426, by affinity chromatography (e.g., U.S. Pat. No. 5,712,383; and PolyATract® mRNA Purification System (Promega Corp., Madison, WI; see Promega's Technical Manual No. TM031), and

by matricies which employ a combination of the above isolation modes (see, e.g. U.S. Pat. No's 5,652,348; *J. Chromatography* 270:117-126 (1983))

One of the first solid phases developed for use in isolating nucleic acids was a specialized resin of porous silica gel particles designed for use in high performance liquid chromatography (HPLC). The surface of porous silica gel particles was functionalized with anion-exchangers which could exchange with plasmid DNA under certain salt and pH conditions. See, e.g. U.S. Pat. No's: 4,699,717, and 5,057,426. Machrey-Nagel Co. (Düren, Germany) was one of the first companies to provide HPLC columns packed with such anion-exchange silica gel particles, and it continues to sell such columns today. See, e.g. Information about NUCLEOGEN® 4000-7DEAE in product information downloaded from the Machrey-Nagel homepage on the Internet on 6/12/98, at http://www.machrey-nagel.com. Each such column was designed so that plasmid DNA bound thereto is eluted in an aqueous solution containing a high concentration of a highly corrosive salt (e.g. plasmid DNA is eluted from the NUCLEOGEN® 4000-7DEAE column in 6 M urea). Each such column had to be washed thoroughly between each isolation procedure to remove the corrosive salt and contaminants bound to the column with the DNA from the system. The nucleic acid solution eluted therefrom also had to be processed further to remove the corrosive salt therefrom before it could be used in standard molecular biology techniques, such as cloning, transformation, digestion with restrictive enzymes, or amplification.

Various silica-based solid phase separation systems have been developed since the early HPLC systems described above. (See, e.g. the silica gel and glass mixture for isolating nucleic acids according to U.S. Pat. No. 5,658,548, and the porous support with silane bonded phase used to isolate oligonucleotides according to U.S. Pat. No. 4,767,670.) Modern silica-based systems utilize controlled pore glass, filters embedded with silica particles, silica gel particles, resins comprising silica in the form of diatomaceous earth, glass fibers or mixtures of the above. Each modern silica-based solid phase separation system is configured to reversibly bind nucleic acid materials when placed in contact with a medium containing such materials in the presence of chaotropic agents. Such solid phases are designed to remain bound to the nucleic acid material while the solid phase is exposed to an external force such as centrifugation or vacuum filtration to separate the matrix and nucleic acid material bound thereto from the remaining media components. The nucleic acid material is then eluted from the solid phase by exposing the solid phase to an elution solution, such as water or an elution buffer. Numerous commercial sources offer silica-

much as nucleic acid samples contaminated with the proteins the proteases are introduced to digest. Specifically, given the proper solution conditions, proteases in a nucleic acid solution will digest any proteins introduced into the solution, including enzymes introduced therein to modify, cut, or transcribe the nucleic acid contained therein for downstream processing or analysis. Protease addition, incubation and removal steps also drive up the cost of nucleic acid isolation, costing time and money compared to isolation systems with no such additional steps.

In all the solid phase systems described above, each solid phase used therein has a substantially uniform surface composition designed to bind to a nucleic acid of interest, in the form of a silica or silica gel surface, or in the form of a silica gel or polymer surface modified with chemical groups exhibiting anion exchanger activities. Bimodal and multimodal systems have also been developed, such as systems: (1) in which multiple columns each of which contains a solid phase modified with a different chemical group from the other columns in the system (e.g., Wheatley J. B., *J. Chromatogr.* (1992) 603: 273); (2) in which a single column is used with a single solid phase with at least two different chemical groups (e.g., Patent '680; Little, E. L. et al., *Anal. Chem.* (1991) 63: 33); or (3) in which two different solid phases are employed in the same column, wherein the two solid phases are separated from one another within the column by solid porous dividers (e.g., U.S. Patent No. 5,660,984). Each of the chemical groups on the surface of the solid supports in the single column or multicolumn multimodal systems is configured to bind to different materials in whatever substrate is introduced into the system. Only a few such bimodal or multimodal column chromatography systems have been developed specifically for nucleic acid isolation (see, e.g. U.S. Pat. No. 5,316,680). Surface group combinations used in such solid phase systems include reverse phase, ion exchange, size exclusion, normal phase, hydrophobic interaction, hydrophilic interaction, and affinity chromatography. Such systems are designed such that only one of the surface groups binds a target species, such as a nucleic acid, while the other surface group(s) bind to and remove one or more non-target species in a mixture.

Bimodal and multimodal systems are far from simple, efficient alternatives to conventional organic or resin methods of nucleic acid isolation described above. Multi-column systems are inherently complex to run, as each column requires a unique set of mobile phase conditions to bind and/or release the desired target or non-target species bound to the stationary solid phase of the system. Non-target species tend to block adjacent

functional groups configured to bind to the target species, thus adversely affecting overall yield. Also, all the bimodal or multimodal systems are only designed to separate a target species from other species for which functional groups have affinity.

At least one mixed mode ion exchange solid phase system has been developed for use in isolating certain types of target compounds, such as proteins or peptides, from an aqueous solution. See U.S. Pat. No. 5,652,348 (hereinafter, "Burton et al. '348") at col. 4, lines 21 to 25. The mixed mode ion exchange system of Burton et al. '348 comprises a solid support matrix with ionizable ligands covalently attached to the sold support matrix. The ionizable ligand is capable of exchanging with and adsorbing the target compound at a first pH and of releasing or desorbing the target compound at a second pH. The ionizable functionality is "either further electrostatically charged or charged at a different polarity at the second pH". (Burton et al. '348, claim 1, col. 25, lines 46-50). The examples of mixed mode ion exchange solid phase systems provided in the Burton et al. '348 patent contain only a single ionizable functionality, an amine residue capable of acting as an anion exchange group at the first pH. The concentration of ionizable ligands present on the solid support matricies disclosed in Burton et al. '348 is sufficiently high to "permit target protein binding at both high and low ionic strength". The only ligand density specifically disclosed and claimed as sufficiently high for the mixed mode ion exchange solid phase of Burton et al. '348 to bind to target proteins at high and low ionic strength is a ligand density which is "greater than the smaller of at least about 1mmol/gram dryweight of resin or at least about 150 μmol/ml of resin" (col 13, lines 22-23; and claim 1). The mixed mode ion exchange system of Burton et al. '348, is specifically designed for use in the isolation of proteins and peptides, not nucleic acids or oligonucleotides.

Materials and methods are needed which can quickly, safely, and efficiently isolate target nucleic acids which are sufficiently free of contaminants to be used in molecular biology procedures. The present invention addresses the need for materials and methods which provide a rapid and efficient means for isolating target nucleic acids from any mixture of target nucleic acids and contaminants, including lysates of gram-negative bacteria, thereby providing purified nucleic acids which can be used in a variety of biological applications, including transfection of cultured cells and *in vivo* administration of nucleic acids to organisms.

BRIEF SUMMARY OF THE INVENTION

Briefly, in one aspect, the present invention is a pH dependent ion exchange matrix designed for use in isolating a target nucleic acid by adsorbing to the target nucleic acid at an adsorption pH and by releasing the target nucleic acid at a desorption pH which is higher than the adsorption pH.

In one embodiment of the present invention, the pH dependent ion exchange matrix comprises a solid support and a plurality of first ion exchange ligands, wherein each first ion exchange ligand comprises:

a cap comprising an amine with a pK of less than about 9, wherein the amine is selected from the group consisting of a primary, a secondary, or a tertiary amine;

a spacer covalently attached to the cap, the spacer comprising a spacer alkyl chain with an amine terminus, and an acidic moiety covalently attached to the spacer alkyl chain; and

a linker comprising a linker alkyl chain covalently attached to the solid support at a first end of the linker alkyl chain and covalently attached to the amine terminus of the spacer at a second end of the linker alkyl chain.

In another embodiment, the present invention is a bimodal pH dependent ion exchange matrix having the same basic structure as the matrix described above except that the spacer does not include an acidic moiety, wherein the bimodal pH dependent ion exchange matrix further comprises a plurality of second ion exchange ligands covalently attached to the solid support. Each second ion exchange ligand comprises an alkyl chain with an acidic substituent covalently attached to the alkyl chain.

In another aspect, the present invention is a method of isolating a target nucleic acid using a pH dependent ion exchange matrix, according to steps comprising:

(a) providing the pH dependent ion exchange matrix;

(b) combining the matrix with a mixture comprising the target nucleic acid and at least one contaminant;

(c) incubating the matrix and mixture at an adsorption pH, wherein the target nucleic acid adsorbs to the matrix, forming a complex;

(d) separating the complex from the mixture; and

(e) combining the complex with an elution solution at a desorption pH, wherein the target nucleic acid is desorbed from the complex.

In yet another aspect, the present invention is a method of making a pH dependent ion exchange matrix, comprising the steps of:

(a) providing a solid phase;

(b) providing a linker comprising a linker alkyl chain having a first end and a second end;

(c) combining the solid phase and the linker under conditions where a covalent bond is formed between the first end of the linker alkyl chain and the solid phase, thereby forming a linker-modified solid phase;

(d) providing an alkyl amine comprising:

a cap comprising an amine with a pK of less than about 9, wherein the amine is selected from the group consisting of a primary, secondary, or tertiary amine;

a spacer which is covalently attached to the cap, wherein the spacer comprises a spacer alkyl chain with an amino terminus, and an acidic substituent covalently attached to the spacer alkyl chain; and

(e) combining the linker-modified solid phase with the alkyl amine under conditions where a covalent bond is formed between the amino terminus of the spacer alkyl chain and the second end of the linker.

In yet another embodiment, the present invention is a method of making a pH dependent ion exchange matrix, according to the steps comprising:

(a) providing a solid support;

(b) providing a first ion exchange ligand comprising:

a cap comprising an amine with a pK of less than about 9, wherein the amine is selected from the group consisting of a primary, secondary, or tertiary amine;

a spacer covalently attached to the cap, the spacer comprising a spacer alkyl chain with an amine terminus, an acidic substitutent covalently attached to the spacer alkyl chain, and a protecting group covalently attached to the acidic substituent; and

specifically includes stationary phases in liquid chromatography (LC), high pressure liquid chromatography (HPLC), particulate matrices embedded into or bound to filters, and magnetic or non-magnetic porous matrix particles which interact with solutes when added directly to a solute mixture.

The term "silica gel" as used herein refers to chromatography grade silica gel, a substance which is commercially available from a number of different sources. Silica gel is most commonly prepared by acidifying a solution containing silicate, e.g. by acidifying sodium silicate to a pH of less than 11, and then allowing the acidified solution to gel. See, e.g. silica preparation discussion in Kurt-Othmer Encyclopedia of Chemical Technology, Vol. 21, 4th ed., Mary Howe-Grant, ed., John Wiley & Sons, pub., 1997 , p. 1021.

The term "glass particles" as used herein means particles of crystalline or vitreous silicas, even though crystalline silicas are not formally "glasses" because they are not amorphous, or particles of glass made primarily of silica. The term includes quartz, vitreous silica, controlled pore glass particles, and glass fibers.

As used herein, the term "silica magnetic particles" refers to silica based solid phases which are further comprised of materials which have no magnetic field but which form a magnetic dipole when exposed to a magnetic field, i.e., materials capable of being magnetized in the presence of a magnetic field but which are not themselves magnetic in the absence of such a field.

The term "magnetic" as used to refer to silica magnetic particles includes materials which are paramagnetic or superparamagnetic materials. The term "magnetic", as used herein, also encompasses temporarily magnetic materials, such as ferromagnetic or ferromagnetic materials. Except where indicated otherwise below, the silica magnetic particles used in this invention preferably comprise a superparamagnetic core coated with siliceous oxide, having a hydrous siliceous oxide adsorptive surface (i.e. a surface characterized by the presence of silanol groups).

The term "surface", as used herein, refers to the portion of the support material of a solid phase which comes into direct contact with a solution when the solid phase is combined therewith.

The term "nucleic acid" as used herein refers to any DNA or RNA molecule or a DNA/RNA hybrid molecule. The term includes plasmid DNA, amplified DNA or RNA fragments, total RNA, mRNA, and genomic DNA.

The term "target nucleic acid" as used herein refers to the particular species of nucleic acid to be isolated in any particular application of the methods or use of the pH dependent ion exchange matrix of the present invention. The target nucleic acid is preferably at least 20 nucleotides long, more preferably at least 100 nucleotides long, and most preferably at least 1,000 nucleotides long.

The solid support component of the pH dependent ion exchange matrix can be made of any common support material, including soft gel supports such as agarose, polyacrylamide, or cellulose, or hard support material such as polystyrene, latex methacrylate, or silica. When the solid phase support material is silica, it is preferably in the form of silica gel, siliceous oxide, solid silica such as glass or diatomaceous earth, or a mixture of two or more of the above. Silica based solid phases suitable for use in the pH dependent ion exchange matrixes of the present invention include the mixture of silica gel and glass described in U.S. Pat. No. 5,658,548, the silica magnetic particles described in PCT Publication Number WO 98/31840, and solid phases sold by Promega Corporation for use in plasmid DNA isolation, i.e. Wizard® Minipreps DNA Purification Resin. Silica gel particles are particularly preferred for use as the solid phase in the pH dependent ion exchange matrix and methods of the present invention. Silica gel particles are stable at much higher pressures than solid phases made from soft gel support material, making the silica gel solid phases suitable for HPLC as well as LC and batch separation applications.

The pH dependent ion exchange matrix used in the present invention is preferably in a form which can be separated from a solute mixture comprising the target nucleic acid and at least one contaminant after the solute mixture is combined therewith, by application of an external force. A skilled artisan would appreciate that the type of external force suitable for use in separating the matrix from the solute mix depends upon the form in which the matrix is presented to the solute mix, and upon the physical properties of the matrix itself. For example, gravity can be used to separate the pH dependent ion exchange matrix from the solute mix when the matrix is in the form of a chromatographic resin loaded on an LC column, when the matrix is in the form of silica particles (e.g., controlled pore glass, silica gel particles, or silica magnetic particles) which are added batch-wise to a solute mixture and then separated therefrom by decantation or filtration, or when the mixed-mode matrix is in the form of a filter with silica particles or chromatographic resin embedded into or attached thereto.

The external force used in the method of isolation is high pressure liquid when the pH dependent ion exchange matrix is the stationary phase of a high pressure liquid chromatography column (HPLC). Other forms of external force suitable for use in the method of this invention include vacuum filtration (e.g. when the solid phase component of the matrix is particles of controlled pore glass, particles of silica gel or silica magnetic particles, or mixtures of one or more of the above types of particles embedded into or attached to a filter), centrifugation (e.g. when the mixed-bed solid phase is particulate), or magnetic (e.g. when the mixed-bed solid phase comprises magnetic or paramagnetic particles).

When the solid phase component of the pH dependent ion exchange matrix is a silica gel particle, it is most preferably a silica magnetic particle. A silica magnetic particle can be separated from a solution using any of the external means described above for use with other types of solid phases, such as those described above. However, unlike the other solid phases, a silica magnetic particle can be separated from a solution by magnetic force, a quick and efficient means of separating a matrix from a solution.

When the solid support component of the pH dependent ion exchange matrix is a silica magnetic particle, the size of the particle is preferably selected as follows. Smaller silica magnetic particles provide more surface area (on a per weight unit basis) for covalent attachment to the plurality of ion exchange ligands, but smaller particles are limited in the amount of magnetic material which can be incorporated into such particles compared to larger particles. The median particle size of the silica magnetic particles used in a particularly preferred embodiment of the present invention is about 1 to 15 μm, more preferably about 3 to 10 μm, and most preferably about 4 to 7 μm. The particle size distribution may also be varied. However, a relatively narrow monodal particle size distribution is preferred. The monodal particle size distribution is preferably such that about 80% by weight of the particles are within a 10 μm range of the median particle size, more preferably within an 8 μm range, and most preferably within a 6 μm range.

The solid support component of the pH dependent ion exchange matrix can be porous or non-porous. When the solid support is porous, the pores are preferably of a controlled size range sufficiently large to admit the target nucleic acid material into the interior of the solid phase particle, and to bind to functional groups or silica on the interior surface of the pores. The total pore volume of a silica magnetic particle, as measured by nitrogen BET method, is preferably at least about 0.2 ml/g of particle mass. The total pore

volume of porous silica magnetic particles particularly preferred for use as components of the pH dependent ion exchange matrix of the present invention, as measured by nitrogen BET, is preferably at least about 50% of the pore volume is contained in pores having a diameter of 600 Å or greater.

Silica magnetic particles may contain substances, such as transition metals or volatile organics, which could adversely affect the utility of target nucleic acids substantially contaminated with such substances. Specifically, such contaminants could affect downstream processing, analysis, and/or use of the such materials, for example, by inhibiting enzyme activity or nicking or degrading the target nucleic acids isolated therewith. Any such substances present in the silica magnetic particles used in the present invention are preferably present in a form which does not readily leach out of the particle and into the isolated biological target material produced according to the methods of the present invention. Iron is one such undesirable at least one contaminant, particularly when the biological target material is a target nucleic acid.

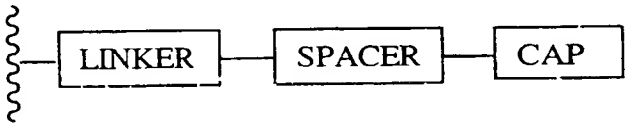
Iron, in the form of magnetite, is present at the core of particularly preferred forms of silica magnetic particles used as the solid phase component of the pH dependent ion exchange matrixes of the present invention. Iron has a broad absorption peak between 260 and 270 nanometers (nm). Target nucleic acids have a peak absorption at about 260 nm, so iron contamination in a target nucleic acid sample can adversely affect the accuracy of the results of quantitative spectrophotometric analysis of such samples. Any iron containing silica magnetic particles used to isolate target nucleic acids using the present invention preferably do not produce isolated target nucleic acid material sufficiently contaminated with iron for the iron to interfere with spectrophotometric analysis of the material at or around 260 nm.

The most preferred silica magnetic particles used in the matrixes and methods of the present invention, siliceous oxide coated silica magnetic particles, leach no more than 50 ppm, more preferably no more than 10 ppm, and most preferably no more than 5 ppm of transition metals when assayed as follows. Specifically, the particles are assayed as follows: 0.33 g of the particles (oven dried @ 110°C) are combined with 20 ml. of 1N HCl aqueous solution (using deionized water). The resulting mixture is then agitated only to disperse the particles. After about 15 minutes total contact time, a portion of the liquid from the mixture is then analyzed for metals content. Any conventional elemental analysis

technique may be employed to quantify the amount of transition metal in the resulting liquid, but inductively coupled plasma spectroscopy (ICP) is preferred.

At least two commercial silica magnetic particles are particularly preferred for use in the matrix of the present invention, BioMag® Magnetic Particles from PerSeptive Biosystems, and the MagneSil™ Particles available from Promega Corporation (Madison, Wisconsin). Any source of magnetic force sufficiently strong to separate the silica magnetic particles from a solution would be suitable for use in the nucleic acid isolation methods of the present invention. However, the magnetic force is preferably provided in the form of a magnetic separation stand, such as one of the MagneSphere® Technology Magnetic Separation Stands (cat. no.'s Z5331 to 3. or Z5341 to 3) from Promega Corporation.

The pH dependent ion exchange matrices of the present invention all include a plurality of first ion exchange ligands covalently attached to a solid phase, according the general structure of formula (I), below:



(I)

wherein the wavy line represents a surface of the solid phase. LINKER comprises a linker alkyl chain, preferably an alkyl chain which includes three (3) to eight (8) carbon atoms. The LINKER preferably also includes at least one additional member selected from the group consisting of oxygen, amine, and carbonyl. The LINKER is preferably an epoxide, such as a glycidyl moiety, or a urea linkage. The SPACER comprises a spacer alkyl chain with an amine terminus, wherein the amine terminus is covalently attached to the LINKER. The other end of the spacer alkyl chain is covalently attached to the CAP. The SPACER alkyl chain can be substituted by at least one sulphur residue. The CAP comprises a primary, secondary, or tertiary amine with a pK value less than 9. The CAP preferably further comprises an aromatic hydrocarbon ring, wherein the amine is either attached to or a member of the ring. When the CAP comprises an aromatic hydrocarbon ring and an amine, the amine is preferably a member of the ring. The CAP more preferably comprises a five or six member aromatic amine ring, such as imidazole or pyridine.

In one embodiment of the present invention, wherein the plurality of first ion exchange ligands are the only ion exchange ligands attached to the solid phase, the

SPACER further comprises a first acidic moiety covalently attached to the spacer alkyl chain. The acidic moiety is preferably a carboxyl residue. In this embodiment of the invention, at least one basic (the amine member of the aromatic hydrocarbon) and at least one acidic moiety are both members of the first ligand. The SPACER is preferably selected from the group consisting of cysteine, alanine, and the alkyl chain portion of a polar amino acid consisting of an alkyl chain and an aromatic hydrocarbon such as histamine and histidine. SPACER and CAP together most preferably define a histamine or a histidine moiety.

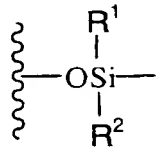
In another embodiment, the present invention is a pH dependent ion exchange matrix comprising a plurality of first ion exchange ligands and a plurality of second ion exchange ligands covalently attached to the same solid support, such as the same silica magnetic particle. The second ion exchange ligand comprises a second alkyl chain and an ion exchange residue covalently attached thereto. The second alkyl chain is preferably an unbranched alkane of one (1) to five (5) carbon atoms. The ion exchange residue is preferably an acidic moiety, more preferably a carboxylic acid. The second ion exchange ligand is most preferably propionate.

In this second embodiment of the pH dependent ion exchange matrix, each first ion exchange ligand can have the same structure as set forth in Formula (I), above, except that the first ion exchange ligand need not have an acidic moiety covalently attached to the spacer alkyl chain when the second ion exchange ligand includes such a moiety. When the second ion exchange ligand includes an acidic moiety, it is preferably a carboxylic acid residue, more preferably a carboxylic acid residue covalently attached to the terminus of the second alkyl chain.

The second type of pH ion exchange matrix described immediately above, hereinafter the "bimodal" ion exchange matrix, preferably has an acidic moiety on one ligand, the second ion exchange ligand, and at least one basic moiety on the other ligand, the amine member of the aromatic hydrocarbon ring component of the first ion exchange ligand. In that preferred configuration, target nucleic acid binding and release capacity of the matrix can be controlled and even fine tuned by varying the relative proportion of first and second ion exchange ligands covalently bound to the solid support. This feature of the bimodal ion exchange matrix makes it particularly desirable for use in the methods of the present invention, although the monomodal ion exchange matrix described above is also well suited for use in the isolation of target nucleic acids according to the present methods.

When the solid phase is silica based, each ion exchange ligand is preferably covalently attached to the solid phase through a silane group, as shown in formula (II), below:

(II)



Wherein, R^1 is selected from the group consisting of -OH, $-OCH_3$, and $-OCH_2CH_3$; and R^2 is represented by the formula $-(OSiR^1_2)_y-R^1$, wherein y is at least 0. When y is zero (0), the ligand is connected to the solid support through a silane monomer. When y is greater than zero, the connection is through a silane polymer.

Target nucleic acids are inherently negatively charged at any pH higher than 2, and can, therefore, reversibly bind to anion-exchangers under solution conditions where ions can be exchanged between the anion-exchanger and the target nucleic acid. The pH dependent ion exchange matrix of the present invention is an anion exchanger at a first pH in which the matrix present is neutral to positively charged. At a second, higher pH the matrix becomes neutral to negatively charged depending on the pK of the acidic moiety of the ion exchange ligand. The target nucleic acid can adsorb to the matrix at the first pH and desorb from the matrix at the second pH. The possible pH range for each of the first and second pH depends upon the nature of the plurality of ion exchange ligands component of the pH dependent ion exchange matrix.

The plurality of ligands include at least one anion-exchange moiety and at least one cation-exchange moiety. The at least one anion-exchange moiety of the pH dependent ion exchange matrix is at least one amine with a pK of less than 9, wherein the amine is selected from the group consisting of a primary, secondary, or tertiary amine. The at least one cation-exchange moiety is an acidic moiety, preferably selected from the group consisting of hydroxyl and carboxyl.

The pH dependent ion exchange solid phase of the present invention is designed for use in the isolation of target nucleic acids. Both the ligand configuration, described above, and ligand density can be adjusted to ensure optimal adsorption and desorption of a given target nucleic acid. The highest ligand density suitable for use in the matrices of the present invention is 500 µmol per gram of dry weight. The lowest ligand density suitable for use in

the pH dependent ion exchange matrices of the present invention is about 25 µmol/g dry weight. The ligand density in the matrices of the present invention is most preferably between 50 and 200 µmol/g dry weight of solid phase.

The anion exchange moiety and cation exchange moiety of the present matrix vary in charge depending upon solution conditions. In the presence of a solution having a first pH, the basic moiety (i.e., the amine) is positively charged and the matrix is capable of exchanging with the target nucleic acid. In the presence of a solution having a second pH which is higher than the first pH, the acidic moiety has a negative charge and the basic moiety has a neutral charge. The matrix is designed to adsorb the target nucleic acid at the first pH and to desorb the target nucleic acid at a pH which is at least about the second pH. pH conditions necessary to ensure adsorption and desorption of the target nucleic acid to the matrix of the present invention depend upon the salt conditions of the adsorption and desorption solutions, and upon the specific composition and density of the plurality of ligands attached to the solid phase. Specifically, the first pH, at which desorption takes place, is preferably between pH 6 and 8 when the ionic strength of the solution is preferably no higher than about 1 M salt, more preferably no higher than about 500mM salt, and most preferably no higher than about 50 mM salt.

The method of isolating a target nucleic acid of the present invention can employ either type of pH dependent ion exchange matrix of the present invention described above alone, or a mixed bed of a pH dependent ion exchange matrix and another type of matrix capable of binding and releasing the target nucleic acid under a different set of solution conditions such as is described in the concurrently filed U.S. Patent Application No. 09/312,139 for MIXED BED SOLID PHASE AND ITS USE IN THE ISOLATION OF NUCLEIC ACIDS.

The present method comprises the steps of providing the pH dependent ion exchange matrix to be used in the method, providing a mixture comprising the target nucleic acid and at least one contaminant, combining the mixture and the matrix at a first pH under conditions where the target nucleic acid adsorbs to the matrix to form a complex, separating the complex from the mixture, and desorbing the target nucleic acid from the complex by combining the complex with an elution solution at a desorption pH. The exact solution conditions necessary to ensure adsorption and desorption of the target nucleic acid to the matrix vary depending upon several factors, including the nature of the target nucleic acid (e.g., RNA or DNA, molecular weight, and nucleotide sequence composition), the pKa

diameter), median particle size of 5.5µm, and iron leach of 2.0 ppm. Specifications of glass particles used in the examples below are provided below.

One skilled in the art of the present invention will be able to use the teachings of the present disclosure to select and use solid supports other than the three silica based solid supports used to make the pH dependent ion exchange particles whose synthesis and use is illustrated in the Examples below. The Examples should not be construed as limiting the scope of the present invention. Other pH dependent ion exchange matrixes, and methods of using the matrixes to isolate target material according to the present invention will be apparent to those skilled in the art of chromatographic separations and molecular biology.

EXAMPLES

The following examples are given to illustrate various aspects of the invention, without limiting the scope thereof:

EXAMPLE 1 - GEL ELECTROPHORESIS

Samples of target nucleic acids isolated according to procedures described in Examples below were analyzed for contamination with non-target nucleic acids, and for size as follows. The samples were fractionated on an agarose gel of appropriate density (e.g., a 1.0% agarose gel was used to analyze plasmid DNA, while a 1.5% agarose gel was used to analyze RNA). The fractionated nucleic acid was visualized using a fluorescent label or by dying the gel with a DNA sensitive stain, such as ethidium bromide or silver staining. The resulting fractionated, visualized nucleic acid was either photographed or visualized using a fluorimager and the resulting image printed out using a laser printer.

In some cases, size standards were fractionated on the same gel as the target nucleic acid, and used to determine the approximate size of the target nucleic acid. In every case where a gel assay was done, the photograph or fluorimage of the fractionated nucleic acid was inspected for contamination by non-target nucleic acids. For example, images of fractionated samples of plasmid DNA were inspected for RNA, which runs considerably faster than DNA on the same gel, and for chromosomal DNA, which runs considerably slower than plasmid DNA on the same gel. Images of isolated plasmid DNA were also inspected to determine whether most of the plasmid DNA shown in the image is intact, supercoiled plasmid DNA.

EXAMPLE 2 – ABSORPTION SPECTROPHOTOMETRY

Samples of target nucleic acids isolated from various media, as described below, were also analyzed using absorption spectrophotometry. Absorption measurements were taken at wavelengths of 260, 280, and 230 nanometers (nm). A_{260}/A_{280} absorption ratios were computed from the measurements. An A_{260}/A_{280} of greater than or equal to 1.80 was interpreted to indicate the sample analyzed therein was relatively free of protein contamination. The concentration of nucleic acid in each sample was determined from the absorption reading at 260 nm (A_{260}).

EXAMPLE 3 - SYNTHESIS OF POROUS SILICA MAGNETIC pH DEPENDENT ION EXCHANGE PARTICLES

Various pH dependent ion exchange ligands were attached to porous silica magnetic particles, according to the following procedures. The silica magnetic pH dependent ion exchange particles synthesized as described herein were used to isolate target nucleic acids, as described in subsequent Examples, below.

A. Preparation of Glycidyl Modified Silica Magnetic Particles

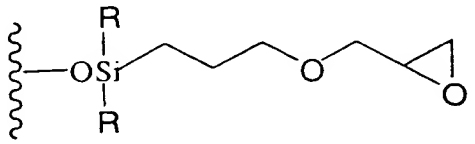
1. Silica magnetic particles were activated by heating under vacuum at 110°C overnight.

2. 10 g of the activated particles were suspended in 100 ml of toluene in a flask, and 3.2 ml of 3-glycidylpropyl-trimethoxysilane was added thereto.

3. The flask containing the mixture was fitted with a condenser and the reaction was refluxed for 5 hr. After cooling to room temperature, the reaction mixture sat for 48 hr at room temperature.

4. The reaction mixture was then filtered and the retentate, including glycidyl-modified silica magnetic particles produced in the reflux reaction, were washed with toluene (2 x 100 ml), hexanes (2 x 100 ml) and ethyl ether (1 x 150 ml). The washed product was then left to dry in the air.

5. A small portion of the product was further dried in a 110°C oven and submitted for elemental analysis. The results (%C 0.75; %H 0.58) are consistent with glycidyl modification of silica gel particles, as illustrated in Formula (III), below. The wavy line in this and other formulae depicted herein and in the remaining Examples below represents the surface of a solid phase, a porous silica magnetic particle in this particular Example.



(XVI)

wherein, R is –OH, OCH_3, or $–OCH_2CH_3$.

6. The glycidyl-modified silica magnetic particles produced as described above were then further modified by the linkage of an amino acid, such as histidine, alanine, or cysteine to the particles, by reaction with the terminal ring of the glycidyl moiety, as described below.

B. Synthesis of Glycidyl-Histidine Modified Silica Magnetic Particles

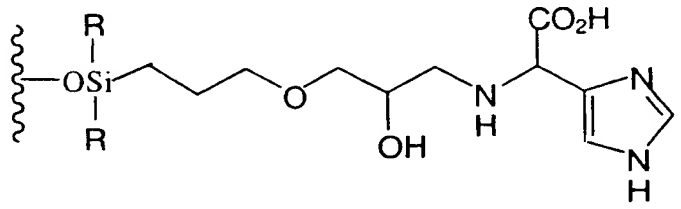
1. 2.0 g. of D,L-histidine was dissolved in a mixture of 20 ml of tetrahydrofuran and 20 ml of water by heating the solution to reflux.

2. To this solution, 2 g of glycidyl-modified silica magnetic particles was added and the resulting suspension was refluxed overnight (18 hr).

3. After cooling to room temperature the reaction mixture was filtered, and the retentate, which included glycidyl-histidine modified silica magnetic particles, was washed once with 100 ml of acetone, three times with 150 ml of water, and once with 150 ml of ether. The solid was air dried.

4. A small portion of the dried solid from step 3 was further dried at 110°C and submitted for elemental analysis. Results: %C 1.35; %H 0.68; %N 0.50. This results are consistent with glycidyl-histidine linkage, such as is as shown in Figure (XVII), below:

(XVII)



wherein, R is –OH, OCH_3, or $–OCH_2CH_3$.

C. Synthesis of Glycidyl -Alanine Modified Silica Magnetic Particles

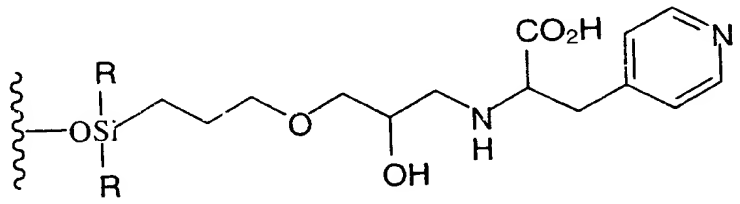
1. 3-(3-pyridyl)-D-alanine (1g) was dissolved in 20 ml of water.

2. To this solution 2 g. of glycidyl-modified silica magnetic particles were added, and the resulting mixture was refluxed overnight.

3. After cooling, the reaction mixture was filtered and washed twice with water, and once with ethyl ether.

4. Elemental analysis of a sample of the product from step 3 showed: %C 0.98; %H 0.56; %N 0.20. This result is consistent with glycidyl-alanine modification, as illustrated in formula (XVIII), below:

(XVIII)



wherein, R is –OH, OCH_3, or $-OCH_2CH_3$.

D. Synthesis of Glycidyl -Cysteine Modified Silica Magnetic Particles

1. 1 g of S-[2-(4-Pyridyl)ethyl]-L-cysteine was suspended in 20 ml of water, and heated to dissolve the material.

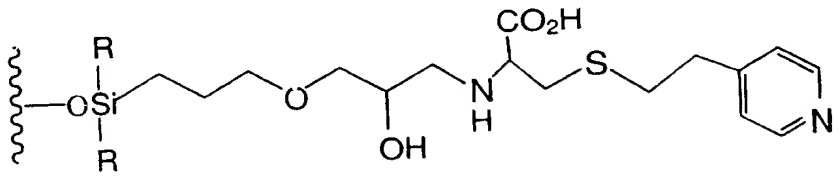
2. To this solution 2.5 g of glycidyl-modified silica magnetic particles were added, and the resulting mixture was refluxed overnight.

3. After cooling the reaction mixture was filtered and washed three times with water and ethyl ether. The material was air dried.

4. Elemental analysis of the material from step 3 showed: %C 1.08; %H 0.42; %N 0.16. This results are consistent with glycidyl-cysteine modification of silica magnetic particles, as

according to formula (XIX), below:

(XIX)



wherein, R is –OH, -OCH_3, or $-OCH_2CH_3$.

EXAMPLE 4 - SYNTHESIS OF NON-POROUS MAGNESIL, GLASS FIBER, AND SILICA GEL GLYCIDYL-LINKED pH DEPENDENT ION EXCHANGE SOLID PHASES

A. Synthesis of Glycidyl-Histidine Modified Non-Porous Silica Magnetic

1. Glycidyl Modification: 6 ml of non-porous silica magnetic particles (Part No. SMR22-552, provided by W.R. Grace) were suspended in 6 ml of toluene, and 0.7 ml of 3-Glycidylpropyltrimethoxysilane was added to the suspension. The resulting mixture was placed on a roto-evaporator and allowed to react overnight. The reaction mixture was filtered and the retentate, including the modified silica magnetic particle product, was washed once with 20 ml of methylene chloride and once with 20 ml of ethyl ether. The product was dried under vacuum in a desiccator over phosphorous pentoxide. Elemental analysis showed: %C 0.3; %H 0.63. This result is consistent with glycidyl modification, as shown in formula (XVI), above.

2. Histidine Linkage: 0.5 g of D,L-histidine was dissolved in a mixture of 4 ml of tetrahydrofuran and 6 ml of water. 1.2 g of glycidyl-modified silica magnetic particles was added to the mixture; and the resulting suspension was refluxed for 5 hr. After cooling to room temperature the reaction mixture was filtered, the solid washed once with 50 ml of methanol and 50 ml. of ethyl ether. The product was dried under vacuum in a desiccator over phosphorous pentoxide. Elemental analysis revealed: %C 0.44; %H 0.64; %N 0.0. This result is consistent with glycidyl linkage of histidine to the non-porous silica magnetic particles, according to formula (XVII), above.

B. Synthesis of Glycidyl-Histidine Modified Glass-Fibers

1. Glycidine Modification: 0.7 g of glass fiber filters (Ahlstrom-122; Ahlstrom Filtration, Inc., Helsinki, Finland.) were suspended in 15 ml of toluene, and 1.0 ml of 3-glycidylpropyltrimethoxysilane was added to the suspension. The resulting mixture was incubated at room temperature for 48 hr. The solution was removed from the resulting modified glass fiber filter products by pipetting. The filter products were washed twice with 30 ml of methylene chloride, then soaked in methylene chloride for 30 min, and washed two more times with 30 ml. each of methylene chloride. This process of soaking and washing was repeated. The filters were dried under vacuum on a roto-evaporator.

2. Histidine Linkage: 0.6 g of D,L-histidine was dissolved in a mixture of 10 ml of tetrahydrofuran and 15 ml of water. This solution was added to the filters and the resulting

suspension was refluxed for 20 hr. After cooling to room temperature the liquids were removed from the reaction by pipetting and the filters were washed extensively with water and with methanol. The washed filters were air dried overnight. Elemental analysis of the end product showed: %C 0.55; %H 0.16; %N 0.0. These results are consistent with glycidyl-histidine linkage, according to formula (IV), above.

C. Synthesis of Glycidyl-Histidine Modified Silica Gel

1. Glycidine Modification: 10.0 g of Silica Gel 110HP [Chromatographic Silica Grade 110HP from W.R. Grace (Baltimore, MD)] was suspended in 45 ml of toluene, and 5.0 ml of 3-glycidylpropyl-trimethoxysilane was added to the suspension. The resulting mixture was placed on a roto-evaporator overnight. The reaction mixture was filtered and the solid product was washed once with 20 ml of methylene chloride and once with 20 ml of ethyl ether. The product was dried under vacuum in a desiccator over phosphorous pentoxide. Elemental analysis: %C 7.75; %H 1.67. These results are consistent with glycidine modification.

2. Histidine Linkage: 10 g of all of the above modified silica was suspended in 30 ml of tetrahydrofuran and 50 ml of water. To this solution 3.8 g of D,L Histidine was added and the resulting suspension was refluxed overnight (about 18 hr). After cooling to room temperature the reaction mixture was filtered, washed once with 200 ml of methanol and once with 50 ml of ethyl ether. The resulting product was dried under vacuum in a desiccator over phosphorous pentoxide. Elemental analysis revealed: %C 9.88; %H 1.92; %N 1.68. These results are consistent with glycidyl-histidine modification, according to formula (IV), above.

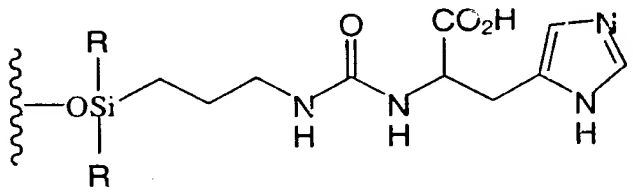
EXAMPLE 5 - PREPARATION OF POROUS SILICA MAGNETIC UREA-LINKED pH DEPENDENT ION EXCHANGE PARTICLES

A. Silica Magnetic Particles Linked to Histidine Through Urea

1. Modification with Urea: 5 g of histidine ethyl ester dihydrochloride was suspended in 50 ml of chloroform and 4.0 ml of triethylamine. 4.8 g of 3-isocyanatopropyl-trimethoxysilane was added to this solution drop-wise, via an addition funnel, and the resulting silane/chloroform solution was stirred overnight. 2.0 g of porous silica magnetic particles were suspended in 25.0 ml of the silane/chloroform solution, and this mixture was placed on a roto-evaporator for 20 hr. The resulting reaction mixture was filtered, and the

retentate, which included silica magnetic particles modified in the reaction, was washed once with 50 ml of chloroform and once with 50 ml of ethyl ether. The washed product was dried in a desiccator under vacuum over phosphorous pentoxide. Elemental analysis revealed: %C 2.38; %H 0.96; %N 0.81. These results are consistent with results one would expect from a silica magnetic particles modified with urea.

2. 1.0 g of the modified particles was suspended in 5% HCl and stirred for 4 hr. The particles were separated from the HCl solution, washed with water, resuspended in 25 ml of water, and filtered. The retentate, which included the modified silica magnetic particles, was washed once with 50 ml of water, once with 50 ml of methanol, and once with 50 ml of ethyl ether. The washed solid was dried under vacuum in a desiccator over phosphorous pentoxide. Elemental analysis showed: %C 1.59; %H 0.84; %N 0.55. These results are consistent with what one would expect from a silica magnetic particle linked to histidine via urea, as illustrated in formula (XX), below:



(XX)

wherein, R is –OH, -OCH_3, or –OCH_2CH_3.

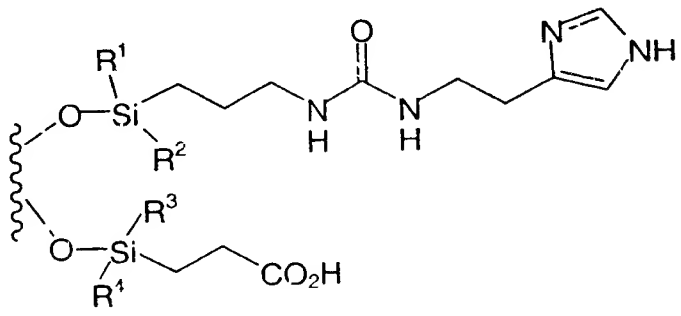
B. Synthesis of Silica Magnetic Particles Linked to Histamine and Propionate

1. Synthesis of N-2-(4-Imidazole)-ethyl-N'-3-propyltriethoxysilylurea: 4.5 g of histamine was suspended in 50 ml of Chloroform. 9.8 g. of 3-Isocyanatopropyltrimethoxysilane was added drop-wise to the suspension, via an addition funnel, and the resulting reaction stirred overnight. After this period the reaction was evaporated to dryness. The product was not further purified. Results of analysis of this intermediate product using nuclear magnetic resonance spectroscopy (NMR) were consistent with what one would expect from N-2-(4-Imidazole)-ethyl-N'-3-propyltriethoxysilylurea. Specifically, NMR (CD3OD) results found were: 7.6 ppm (s, 1H); 6.8 (s, 1H); 4.7 (broad s, 4H); 3.8 (q, 4H); 3.6 (q, 1H) 3.36 (t, 2H); 3.30 (m, 1H); 3.07 (t, 2H); 2.72 (t, 2H); 1.55 (m, 2H); 1.2 (m, 6H).

2. Linkage of Histamine via Urea: 1.0 g of silica magnetic particles was suspended in 10 ml of chloroform, and 1.2 g of the N-2-(4-Imidazole)-ethyl-N'-3-propyltriethoxysilylurea produced in step 1, above, was added to the suspension. The resulting mixture was placed on a roto-evaporator for 48 hr. The reaction was filtered and resuspended in 40 ml of Chloroform. The solid was filtered and washed with chloroform and ethanol. The solid was dried in a desiccator under vacuum over phosphorous pentoxide for 2hr. Elemental analysis results (%C 5.46; %H 1.16; %N 2.35) were consistent the results one would expect to obtain from silica magnetic particles modified with histamine.

3. Methyl Propionate Modification: 1 g of the entire amount of histamine modified silica magnetic particles from step 2, above, was suspended in 10 ml of toluene and 1.0 ml of 2-(carbomethoxy)ethyltrichlorosilane was added drop-wise with stirring. The resulting reaction mixture stirred for 2hr. After this time the solid was filtered and washed with chloroform and ethanol. The product was dried under vacuum for 1 hr in a desiccator over phosphorous pentoxide. Elemental analysis results (%C 7.24; %H 1.52; %N 2.07) were consistent with methyl propionate modification of histamine modified particles.

4. Removal of Methyl Group from the Propionate Residues: 1 g of silica magnetic particles modified in Step 3 was suspended in 5% HCl and stirred for 4 hrs. The reaction products were separated from the solution by filtration. The retentate of reaction product, which included the modified particles, was washed with water and methanol. The washed product was dried under vacuum in a desiccator over phosphorous pentoxide. Elemental analysis results (%C 6.14; %H 1.37; %N 1.47) were consistent with silica magnetic particles linked to histamine through urea and also modified by propionate, according the formula (XXI), below:



(XXI)

wherein, R^1 and R^3 are, independently, -OH, $-OCH_3$, or $-OCH_2CH_3$; R^2 is $-(OSiR^2_2)_y-R^2$, wherein y is at least 0; and R^4 is $-(OsiR^3_2)_z-R^3$, wherein z is at least 0.

C. Synthesis of Silica Magnetic Particles Linked to Histidine and Propionate

1. Histidine was covalently attached to silica magnetic particles via a urea linkage, using a procedure similar to that used to attach histamine in part A of this Example, above.

2. The same final two steps used to covalently attach propionate to the urea-linked histamine particles in part B of the Example, above were used to covalently attach propionate to the silica magnetic particles linked to histidine via propionate.

EXAMPLE 6 - PREPARATION OF CLEARED LYSATE OF PLASMID DNA

E. coli bacteria cells, DH5α strain, were transformed with pGL3-Control Vector (Promega) plasmid DNA, and grown in an overnight culture of Luria Broth ("LB") medium at 37°C, then harvested by centrifugation.

The following solutions were used to prepare a lysate of the harvested cells, as described below:

Cell Resuspension Solution:

50mM Tris-HCl, pH 7.5

10mM EDTA

100µg/ml DNase-free ribonuclease A (RNase A)

Wizard® Neutralization Buffer (Promega Corp.):

1.32M KOAc (potassium acetate), pH 4.8

Cell Lysis Solution:

0.2M NaOH

1% SDS (sodium dodecyl sulfate)

A cleared lysate of the transformed cells was produced as follows:

1. The cells from 1 to 10ml of bacteria culture were harvested by centrifuging the culture for 1-2 minutes at top speed in a microcentrifuge. The harvested cells were resuspended in 250µl of Cell Resuspension Solution, and transferred to a microcentrifuge tube. The resulting solution of resuspended cells was cloudy.

2. 250µl of Cell Lysis Solution was then added to the solution of resuspended cells and mixed by inversion until the solution became relatively clear, indicating the resuspended cells had lysed.

3. 350µl of Wizard® Neutralization Buffer was added to the lysate solution, and mixed by inversion. The lysate became cloudy after the Neutralization Solution was added.

4. The solution was then spun in a microcentrifuge at top speed (about 12,000 G) for 10 minutes to clear the lysate.

EXAMPLE 7 - ISOLATION OF PLASMID DNA USING POROUS SILICA MAGNETIC GLYCIDYL-HISTIDINE pH DEPENDENT ION EXCHANGE PARTICLES

All preps were processed in 1.5ml tubes, and all steps were performed at room temperature:

1. The cleared lysate from step 5 of Example 6 was transferred to a clean 1.5 ml tube containing 150ul of an pH dependent porous silica magnetic ion exchange particles (15 mg of particles) linked to histidine through a glycidyl moiety, wherein the particles prepared as described in Example 3B. The resulting mixture of particles and solution was vortexed, and incubated at room temperature for 5 minutes.

2. The silica magnetic ion exchange particles contained in the tube were held against the inner side-wall of the tube by magnetic force, while the tube cap and side-wall were washed with the lysate solution four times by inversion, and allowed to sit for 1 minute at room temperature. The solution was removed and discarded.

3. The particles tube and cap were washed with 1.0 ml nanopure water.

4. Magnetic force was used to hold the silica magnetic particles in the tube while liquid in the tube was removed therefrom and from the tube cap. The liquid was discarded.

5. The particles were resuspended by vortexing in 300µl of 66mM potassium acetate and 800mM NaCl (pH 4.8). Step 3 was repeated.

6. Step 5 was repeated three times, for a total of four salt washes.

7. The silica magnetic particles remaining in the tube were resuspended in 1.0 ml of nanopure water.

8. The silica magnetic ion exchange particles were separated from the water by magnetic force. The tube cap and side-wall was washed with water by tube inversion (4X), and allowed to sit 1 minute.

9. Liquid was removed from the tube and cap.

10. Steps 7-9 were repeated for a total of 2 washes, with water.

11. 100ul of 10mM Tris pH 8.0 was added to the tube to elute the DNA, and the tube was vortexed thoroughly.

12. The silica magnetic ion exchange particles were separated from the eluent by magnetic force, and the eluent removed to a clean tube.

Analytical analysis of the eluent from step 12 showed that plasmid DNA was obtained which was relatively free of contaminating proteins or other nucleic acids. Specifically, analysis of the eluent using gel electrophoresis according to the procedure set forth in Example 1, above, showed no RNA or chromosomal RNA contamination. Analysis of the eluent using absorption spectroscopy as described in Example 2, showed the yield of pGL-3 plasmid DNA to be 30µg. Absorbance ratio results (A_{260}/A_{280} ratio of 1.84) indicated the plasmid DNA isolated according to the procedure described above was free of protein contamination.

EXAMPLE 8 - ISOLATION OF PLASMID DNA FROM A CLEARED LYSATE USING GLYCIDYL-HISTIDINE GLASS FIBERS

A cleared lysate from 5 ml of an overnight culture of DH5α cells transformed with pGL3 Control Vector plasmid DNA was prepared as described in Example 3. The cleared lysate was added to a column containing 42 mg of Ahlstrom 121 glass fiber modified by glycidyl-histidine, as described in Example 4B, above. After 10 minutes of binding time, the column was centrifuged to remove the alkaline lysate solution. The column was then washed using 700µl of nanopure water, which was removed by column centrifugation. This water wash was repeated twice (for a total of three washes). The DNA was eluted with 100µl of 10 mM Tris pH 8.0, and the solution collected into a 1.5 ml tube by column centrifugation. The eluted DNA was examined by gel electrophoresis according to the procedure set forth in Example 1, and no RNA or chromosomal DNA contamination was detected. Analysis by atomic absorbsion spectroscopy showed a DNA yield of 36 µg, and an A_{260}/A_{280} ratio of 1.86.

The column was washed with 400 µl of 10mM Tris pH 8.0 (which was removed by column centrifugation), and washed again with 2 X 700µl of 100mM Tris, 2.0M NaCl (also removed by column centrifugation). The column was then washed with 700µl of nanopure water, (removed by column centrifugation), and air dried for 12 hours at room temperature.

The column was reused, following the same procedure as outlined above. The resulting DNA again showed no visible RNA by gel electrophoresis, and a DNA yield of 30ug and an A_{260}/A_{280} ratio of 1.84.

EXAMPLE 9 - ISOLATION OF PLASMID DNA FROM A CLEARED LYSATE USING NON-POROUS GLYCIDYL-HISTIDINE ION EXCHANGE PARTICLES FUNCTIONALIZED WITH GLYCIDYL HISTIDINE

A cleared lysate of DH5α cells transformed with pGL3 Control Vector plasmid DNA was prepared as described in Example 6, except 500ul of Wizard® Neutralization Buffer was added to the lysed cells in step 3, rather than 350ul. Plasmid DNA was isolated from the cleared lysate using non-porous glycidyl-histidine silica particles prepared as described in Example 4A, as follows:

The cleared lysate was combined with 15mg of the glycidyl-histidine non-porous silica particles in a 3 ml syringe barrel, and allowed to sit at room temperature for 1 hour.

The lysate was then pushed through the syringe barrel by positive pressure. Two 1.0 ml washes with nanopure water were performed, using positive pressure to remove the liquid. Then 100ul of 10mM Tris, pH 8.0 was used to elute the DNA. The eluted DNA was removed by positive pressure into a clean 1.5 ml tube.

Analysis by gel electrophoresis, according to the procedure of Example 1, showed the eluent to contain supercoiled plasmid DNA, with no evidence of contamination with chromosomal DNA or RNA. Absorption analysis of the eluent, according to the procedure of Example 2, showed a yield of 10mg of DNA, and an absorbance ratio of A_{260}/A_{280} of 1.61.

EXAMPLE 10 - ISOLATION OF PLASMID DNA FROM A CLEARED LYSATE USING POROUS SILICA MAGNETIC GYLCIDYL-ALANINE

Plasmid DNA was isolated from DH5α *E. coli* bacteria cells transformed with pGEM-3Zf+ DNA, as follows. Preps were processed in 1.5ml tubes. All steps were performed at room temperature, except where indicated otherwise below.

1. 2.5 ml of Wizard® Resuspension Solution was added to a 50 ml pellet of transformants, and vortexed vigorously to resuspend cells.
2. 265μl of resuspended cells were added to two tubes.
3. 250μl of Wizard® Lysis Buffer was added per tube, and gently mixed to avoid sheering genomic DNA.

4. 350μl of Wizard® Neutralization Solution was added per tube, and mixed gently.

5. The tubes were centrifuged at 14k rpm for 10 minutes.

6. The cleared solution was removed and placed in a clean 1.5 ml tube containing 150ul of 100mg/ml (15mg) silica magnetic glycidyl-alanine particles prepared as described in Example 3C, above. The resulting mixture was vortexed, and incubated 5 minutes.

7. The particles were separated from the mixture, using a magnetic separator. The tube caps were washed by tube inversion (4X), and incubated 1 minute.

8. Liquid was removed from tubes, including caps.

9. Tubes were washed with 1.0 ml of nanopure water.

10. Steps 7 and 8 were repeated.

11. Steps 9 and 10 were repeated twice, for a total of 3 washes.

12. An elution buffer of 100μl of 20mM Tris-HCl, pH 9.5, was added to each tube. The particles and buffer were mixed well to allow plasmid DNA which had adsorbed to the particles to elute therefrom.

13. The particles were separated from the resulting eluent by magnetic force. The eluent solution in each tube was transferred to a clean tube.

Duplicate isolations conducted according to the procedure described above yielded 21.7 μg (A260/280 of 1.86) and 16.1μg (A260/280 of 1.89) of plasmid DNA. No RNA was visible by analysis using gel electrophoresis.

EXAMPLE 11 - COMPARISON OF COUNTERION CONDITIONS REQUIRED TO ELUTE PLASMID DNA FROM SILICA MAGNETIC UREA-LINKED HISTAMINE, AND SILICA MAGNETIC UREA-LINKED HISTAMINE AND PROPIONATE BIMODAL ION EXCHANGE PARTICLES AT VARIOUS pH'S

The minimum amount of sodium chloride and a buffer required to elute plasmid DNA from each of two different types of silica magnetic pH dependent ion exchange particles was assayed at each of several different pH's, according to the following procedure. One of the two types of particles used in this assay was silica magnetic particles linked to histidine through a urea residue (referred to in the present Example as "urea-histidine IE particles"), prepared as described in Example 5A, above. The other type of particle used in this Example was silica magnetic particles linked directly to propionate and

linked to histamine through a urea residue (hereinafter, "bimodal-histamine -propionate IE particles") prepared as described in Example 5B, above. Elemental analysis of the bimodal-histamine -propionate IE particles showed 260 μmoles of histamine and 900 μmoles of propionate.

Cleared lysates were prepared from the DH5α strain of *E. coli* bacteria cells transformed with pGL3-Control Vector (Promega), as described in Example 6, above, modified as follows. Cells from 50ml of an overnight culture of the transformants were harvested by centrifugation, and resuspended in 2.5ml of Wizard® Resuspension Solution. The cells were lysed by adding 2.5ml of Wizard®Lysis Solution to the resuspended cells. 3.5 ml of Wizard® Neutralization Solution was added to the resulting lysate. The lysate was cleared by centrifugation, and the supernatant transferred to a sterile 50ml tube.

The urea-histidine IE particles and bimodal-histamine -propionate IE particles were tested and compared to one another for their capacity to bind to and release plasmid DNA from the cleared lysate prepared as described immediately above. The elution solution used to isolate plasmid DNA with each of the two types of particles varied, with a pH ranging between pH 4.2 and 9.5:

1. 700μl of the cleared lysate was added to each 1.5 ml microfuge tube in each of four sets of two samples for each of the two types of particles tested. Each 1.5 ml microfuge tube contained 150μl of either of the two types of particles (15 mg). Each tube was capped and mixed by inversion. The resulting suspension was incubated at room temperature for 5 minutes.

2. The particles and solution were separated by magnetic force, and the solution removed from each tube. 1.0 ml of nanopure water was added to each tube, used to wash the particles, separated from the particles by magnetic force, and removed from the tube. For all the sets of samples except those to be eluted at a pH of below pH 5 (e.g. samples to be eluted at 4.2 or 4.8), the water wash was repeated.

3. The particles were resuspended in 300μl of the putative elution solution. The particles were magnetically separated, and the solution carefully removed to a clean 1.5ml tube. The salt concentration of the elution solution has modified, by addition of either water or 5M NaCl, to a final concentration of 1M NaCl. The DNA (if present) was concentrated by precipitation with 1.0ml of -20°C ethanol. The DNA was pelleted by centrifugation in a

microfuge at 12,000 X g for 10 minutes. The pellets were dried to remove ethanol, and resuspended in 100µl of 10mM Tris HCl pH 9.5.

4. The particles remaining from step 3 were washed once with 1.0 ml nanopure water, and then treated as the particles at the beginning of step 3. In this way, a variety of elution solutions were tested, in a stepwise fashion, using the same DNA bound particles.

5. For elution conditions above pH 8.0, 100µl of 10mM Tris HCl was used in the case of the bifunctional IE particles. Similar testing of the urea-histamine IE particles showed no DNA elution at 10mM Tris HCl, even at pH 9.5. The eluted DNA was examined by gel electrophoresis to determine the minimum counterion concentration need for DNA elution. Once the approximate concentration was determined, the procedure was repeated to confirm the concentration of potassium acetate and NaCl at pH 4.8, and the concentration of Tris HCl and NaCl at pH 7.3, and pHs above 7.3.

Elution conditions used on each set of samples prepared as described above are shown in Table 1, below:

TABLE 1

pH	Urea-Histidine IE Particles	Bifunctional IE Particles
4.2	33mM KOAc / 2.15M NaCl	
4.8		33mM KOAc / 1.7M NaCl
7.3	100mM Tris HCl / 600mM NaCl	100mM Tris / 300mM NaCl
8.0	100mM Tris / 300mM NaCl	100mM Tris / no NaCl
8.7		100ul of 10mM Tris HCl
9.5	100ul of 50mM Tris HCl	

The results above demonstrate that the addition of propionate groups to urea-histidine IE particles reduces the amount of counterion concentration required to elute DNA from such particles.

EXAMPLE 12 - ISOLATION OF PCR AMPLIFIED DNA FROM UNINCORPORATED NUCLEOTIDES AND PRIMERS, USING NON-POROUS SILICA MAGNETIC GLYCIDYL-HISTIDINE pH DEPENDENT ION EXCHANGE PARTICLES. SIMILAR PURIFICATION OF PCR AMPLIFIED DNA USING POROUS SILICA MAGNETIC GLYCIDYL CYSTEINE pH DEPENDENT ION EXCHANGE PARTICLES

The human APC (*Adenomatous Polypoptosis Coli*) gene was amplified in a PCR amplification reaction, wherein human genomic template DNA was added to a reaction mix containing:

40ul 10X AmpliTaq® PCR buffer (no Mg++) [Perkin Elmer];

40ul 25mM $MgCl_2$;

13ul 10mM dNTP mix;

13ul APC primers (50 pmoles/µl), with nucleotide sequences: 5'GGA TCC TAA TAC GAC TCA CTA TAG GAA CAG ACC ACC ATG CAA ATC CTA AGA GAG AAC AAC TGT C3' [SEQ ID NO:1], and 5'CAC AAT AAG TCT GTA TTG TTT CTT 3' [SEQ ID NO:2];

6.4 ul AmpliTaq® [Perkin Elmer]; and

273.6ul of nanopure water [total = 392µl].

The amplification reaction was run for 35 cycles on a Perkin Elmer 4800 thermocycler. A 1.8 kb DNA product was the result of the amplification.

The resulting PCR amplified gene was isolated from other components in the reaction mix, above according to the following isolation procedure:

1. 20µl of the PCR reaction mix was added to 200µl of 66mM KOAc+900mM NaCl, pH 4.8, and mixed. Then, 20 µl (2 mg) of non-porous glycidyl-histidine silica magnetic particles was added.

2. After mixing, the solution was incubated for 5 minutes at room temperature. The particles were separated by use of a magnetic separator, and the solution was removed to a clean 1.5 ml tube.

3. The particles were resuspended by vortexing in 200µl of nanopure water, and separated from the resulting solution. The particles were separated using a magnetic separator, the cap and side-wall of the tube were washed by inverting the tube, and the solution was removed from the cap and tube, and placed in a clean 1.5 ml tube.

4. The PCR amplified DNA was eluted in - µl of 10mM Tris HCl pH 8.0. The particles were separated by magnetic force and the eluted DNA was removed to a clean 1.5 ml tube.

5. Using gel electrophoresis (see Example 1), the solutions obtained from steps 2, 3, and 4 were compared with a sample of the original PCR reaction. The solution from steps 2 showed no visible PCR amplified DNA. The solution from step 2 showed a small amount (about 10% of the initial amount) of the PCR DNA. The solution from step 4 showed an amount of PCR DNA >80% of the initial amount in the reaction mix, and no visible unincorporated primers and nucleotides, as seen in the initial PCR reaction solution.

The same procedure was followed using MagneSil™ (no histidine ligand) porous particles, and resulted in no visible DNA at the end of step 4.

The same amplification mixture was purified using porous silica magnetic glycidyl-cysteine pH dependent ion exchange particles and using silica magnetic particles (as a control), according to the following procedure:

1. Three 1.5 ml tubes were set up with 20ul of amplification mixture mixed with 200ul of 33mM KOAc / 400mM NaCl, pH 4.8. To tubes 1 and 2, 20 µl (2mg) of Mag-IE-glycidyl-cysteine was added and mixed. To tube 3, 20µl of Magnesil™ particles was added and mixed.

2. Each tube was incubated 10 minutes at 20°C, and the particles in each tube separated from the solution in each tube by magnetic force, for 2 minutes.

3. The solution from each tube was removed. The sololutions from tubes 1 and 2 were processed according to steps 4-5, below. The particles in tube 3 were resuspended in 33mM KOAc/ 400mM NaCl, pH 4.8, magnetically separated for 2 minutes, and the solution removed and processed according to steps 4-5, below.

4. The particles were resuspended in 200ul of nanopure water, magnetically separated, and the solution removed from the tube.

5. DNA was eluted in 20ul of 50mM Tris HCl pH 9.5

Aliquots of the original amplification reaction products and of the eluents from Magnesil™ (tube 1, above) and from Mag-IE-glycidyl-histidine (tubes 2-3 above) were analyzed by gel electrophoresis, as described in Example 1, above. The resulting gel was stained with ethidum bromide, and a photograph thereof taken under UV light. Figure 4 shows the gel, with:,

Lane 1: Eluent from the Magnesil™ particles (tube 1, above).

Lane 2: Eluent from the Mag-IE-glycidyl-histidine particles (tube 2, above), with no wash step prior to transfer of the particles from the amplification reaction solution to nanopure water in step 4, above.

Lane 3: Eluent from the Mag-IE-glycidyl-histidine particles (tube 3, above), after washing the particles in 33mM KOAc/400mM NaCl, pH 4.8 prior to transfer to nanopure water in step 4, above.

Lane 4: Aliquot of the amplified DNA reaction mixture.

Note that the amplified DNA reaction mixture includes bands other than the desired amplification product. The Magnesil™ particles appear to have failed to isolate any detectable quantity of the amplified DNA fragments, as no bands are visible in lane 1 of Figure 4. Both isolation procedures with Mag-IE-glycidyl-histidine produced amplified DNA isolated from low molecular weight species (the band below the primary band in lane 4). However, considerably more amplified DNA was produced from tube 2, without the additional wash step, than was isolated from tube 3 with the additional wash step.

EXAMPLE 13: ISOLATION OF HUMAN GENOMIC DNA FROM BUCCAL SWABS, USING NON-POROUS SILICA MAGNETIC GLYCIDYL-HISTIDINE PARTICLES

Genomic DNA was isolated from buccal swabs using non-porous silica magnetic glycidyl-histidine ion exchange particles, synthesized as described in Example 3B, above, as follows:

Tissue samples were obtained from two inner cheek areas of human subjects, using cotton swabs (buccal collection), and the swabs were allowed to sit at room temperature for 10 minutes, with occasional swirling, in 700 µl of a cell lysis buffer (75mM Na Citrate pH 7.0 / 1.5% Tween) in a 1.5 ml microfuge tube. The swabs were removed and the liquid in the swabs was pressed out by running it over the opening of the tube, pressing the swab into the interior side of the tube.

30µl of proteinase K (18mg/ml) was added to each tube, and 50 µl (5 mg) of non-porous silica magnetic glycidyl-histidine particles was added per tube, and mixed well. Samples were incubated at room temperature for 5 minutes, with occasional mixing by tube inversion.

The tubes were placed on a magnetic rack to allow separation of the solution and particles, and the solution was removed from the tube.

The particles were washed twice with 1.0 ml of nanopure water. After removal of the second 1 ml of water, the DNA was eluted in 40µl of 20mM Tris HCl pH 9.5, at 65 °C for 5 minutes.

Magnetic force was used to separate the particles from the eluted DNA.

The eluted DNA was examined by gel electrophoresis, as described in Example 1, above, and compared to a control sample of a known amount of genomic DNA to estimate the quantity of DNA eluted. Each 40 µl sample of eluted DNA was found to contain greater than 100 ng of genomic DNA.

EXAMPLE 14: COMPARISON OF COUNTERION CONDITIONS REQUIRED TO ELUTE PLASMID DNA FROM SILICA MAGNETIC UREA-HISTIDINE pH DEPENDENT ION EXCHANGE PARTICLES AND SILICA MAGNETIC UREA-HISTIDINE PROPIONATE BIMODAL pH DEPENDENT ION EXCHANGE PARTICLES

The minimum amount of sodium chloride and a buffer required to elute plasmid DNA from each of two different types of silica magnetic pH dependent ion exchange particles was determined at each of several pH's, according to the following procedure. Silica magnetic urea-histidine IE particles prepared as described in Example 5A, and silica magnetic bimodal urea-histidine -propionate IE particles prepared as described in Example 5C were used to isolate plasmid DNA from a cleared lysate, as follows.

Cleared lysates were prepared as described in example 11. The procedure for comparing the elution profiles of the two particles was as described in example 11. The pHs tested were 4.8, 7.3, and 9.5. The results obtained are shown in Table 3, below:

TABLE 3

MAGNETIC PARTICLE AND pH CONDITIONS	ELUTION/NON-ELUTION CONDITIONS
Urea-histidine IE particles, pH 4.8	DNA eluted in 33mM KOAc/1.45M NaCl, did not elute in 33mM KOAc / 1.40M NaCl
Bimodal urea-histidine -propionate IE particles, pH 4.8	DNA eluted in 33mM KOAc/0.80M NaCl, did not elute in 33mM KOAc / 0.70M NaCl
Urea-histidine IE particles, pH 7.3	DNA eluted in 100mM Tris HCl, did not elute in 80mM Tris HCl
Bimodal Urea-histidine -propionate IE particles, pH 7.3	DNA eluted in 60mM Tris HCl, did not elute in 50mM Tris HCl
Urea-histidine IE particles, pH 9.5	Did not elute in 100ul of 10mM Tris HCl, but eluted in 100ul of 100mM Tris HCl
Bimodal Urea-histidine -propionate IE particles, pH 9.5	Eluted in 100ul of 10mM Tris HCl

By spectrophotometric analysis, the elutions in 100ul of 10mM Tris HCl at pH 9.5 yielded 30 μg (A_{260}/A_{280} of 1.78) of DNA for the bimodal urea-histidine –propionate IE particles and less than 2 µg of DNA for the urea-histidine IE particles. No DNA was detected on analysis of the eluent from the urea-histidine IE particles, by gel electrophoresis. The results above indicate that the addition of propionate to the urea-histidine particles lowered the needed concentration of counter-ion (chloride) required for elution of the DNA at pH 4.8, 7.3 and 9.5.

CLAIMS

What is claimed is:

1. A pH dependent ion exchange matrix, comprising:

a solid support, and

a plurality of first ion exchange ligands, each first ion exchange ligand comprising:

a cap comprising an amine with a pK of less than about 9;

a spacer covalently attached to the cap, the spacer comprising a spacer alkyl chain with an amine terminus and an acidic moiety covalently attached to the spacer alkyl chain; and

a linker comprising a linker alkyl chain covalently attached to the solid support at a first end of the linker alkyl chain and covalently attached to the amine terminus of the spacer at a second end of the linker alkyl chain;

wherein the matrix has a capacity to adsorb to a target nucleic acid at a first pH, and to release the target nucleic acid at a desorption pH which is higher than the first pH.

2. The matrix of claim 1, wherein the solid support is a silica based material.

3. The matrix of claim 2, wherein the silica based material is a glass fiber.

4. The matrix of claim 2, wherein the silica based material is a silica gel particle.

5. The matrix of claim 4, wherein the silica gel particle is paramagnetic.

6. The matrix of claim 4, wherein the silica gel particle is porous.

7. The matrix of claim 4, wherein the silica gel particle is non-porous.

8. The matrix of claim 1, wherein the cap further comprises an aromatic hydrocarbon ring.

9. The matrix of claim 8, wherein at least one member of the aromatic hydrocarbon ring is the amine with a pK of less than about 9.

10. The matrix of claim 9, wherein the aromatic hydrocarbon ring is selected from the group consisting of pyridine, and imidazole.

11. The matrix of claim 1, wherein the amine with a pK of less than 9 has a pK of at least about 4 and up to about 6.

12. The matrix of claim 1, wherein the acidic moiety is selected from the group consisting of hydroxyl, carboxyl, and carbonyl.

13. The matrix of claim 1, wherein the spacer alkyl chain comprises two (2) to five (5) carbon atoms.

14. The matrix of claim 1, wherein the spacer is selected from the group consisting of cysteine and alanine.

15. The matrix of claim 1, wherein the aromatic hydrocarbon covalently linked to the spacer define a basic amino acid moiety selected from the group consisting of histidine and histamine.

16. The matrix of claim 1, wherein the linker alkyl chain comprises three (3) to eight (8) carbon atoms.

17. The matrix of claim 1, wherein the linker alkyl chain includes at least one member selected from the group consisting of oxygen and amine.

18. The matrix of claim 1, wherein the linker is selected from the group consisting of: glycidine and urea.

19. The matrix of claim 1, wherein the matrix is an anion exchanger capable of exchanging with the target nucleic acid at the first pH, and the matrix has a net neutral or negative charge at the desorption pH.

20. The matrix of claim 1, wherein the desorption pH is at least about 4.0 and up to about pH 10.0.

21. The matrix of claim 1, wherein the matrix can be reused through at least two cycles of adsorption of the target nucleic acid to the matrix at the first pH and of release from the matrix at the desorption pH.

22. A pH dependent ion exchange matrix for isolating a target nucleic acid, comprising:
a silica magnetic particle; and
a plurality of first ion exchange ligands, each first ion exchange ligand comprising:
an aromatic hydrocarbon ring, wherein at least one member of the ring is an amine with a pK of less than about 9;
a spacer covalently attached to the aromatic hydrocarbon ring, the spacer comprising a spacer alkyl chain of with an amine terminus, and an acidic moiety covalently attached to the spacer alkyl chain; and
a linker comprising a linker alkyl chain covalently attached to the silica magnetic particle through a silica residue at a first end of the linker alkyl chain and covalently attached to the amine terminus of the spacer at a second end of the linker alkyl chain;
wherein the matrix has a capacity to adsorb to a target nucleic acid at a first pH, and to release the target nucleic acid at a desorption pH which is higher than the first pH.

23. The matrix of claim 22, wherein the cap further comprises an aromatic hydrocarbon ring.

24. The matrix of claim 23, wherein at least one member of the aromatic hydrocarbon ring is the amine with a pK of less than about 9.

25. The matrix of claim 24, wherein the aromatic hydrocarbon ring is selected from the group consisting of pyridine, and imidazole.

26. The matrix of claim 22, wherein the amine with a pK of less than 9 has a pK of at least about 4 and up to about 6.

27. The matrix of claim 22, wherein the acidic moiety is selected from the group consisting of hydroxyl, carboxyl, and carbonyl.

28. The matrix of claim 22, wherein the spacer alkyl chain comprises two (2) to five (5) carbon atoms.

29. The matrix of claim 22, wherein the spacer is selected from the group consisting of cysteine and alanine.

30. The matrix of claim 22, wherein the aromatic hydrocarbon covalently linked to the spacer define a basic amino acid moiety selected from the group consisting of histidine and histamine.

31. The matrix of claim 22, wherein the linker alkyl chain comprises three (3) to eight (8) carbon atoms.

32. The matrix of claim 22, wherein the linker alkyl chain includes at least one member selected from the group consisting of oxygen and amine.

33. The matrix of claim 22, wherein the linker is selected from the group consisting of: glycidine and urea.

34. The matrix of claim 22, wherein the matrix is an anion exchanger capable of exchanging with the target nucleic acid at the first pH, and the matrix was a net neutral or negative charge at the desorption pH is not.

35. The matrix of claim 22, wherein the matrix can be reused through at least two cycles of adherence of the target nucleic acid to the matrix at the first pH and release from the matrix at the desorption pH.

36. A multimodal pH dependent ion exchange matrix, comprising:

a solid support;

a plurality of first ion exchange ligands, each first ion exchange ligand comprising:

a cap comprising an amine with a pK of less than about 9;

a spacer covalently attached to the cap, the spacer comprising a spacer alkyl chain with an amine terminus, and

a linker comprising a linker alkyl chain covalently attached to the solid support at a first end of the linker alkyl chain and covalently attached to the amine terminus of the spacer at a second end of the linker alkyl chain;

a plurality of second ion exchange ligands, each second ion exchange ligand comprising:

a second alkyl chain; and

a second acidic moiety covalently attached to the second alkyl chain,

wherein the matrix has a capacity to adsorb to a target nucleic acid at a first pH, and to release the target nucleic acid at a desorption pH which is higher than the first pH.

37. The matrix of claim 36, wherein the solid support is a silica based material.

38. The matrix of claim 37, wherein the silica based material is a silica magnetic particle.

39. The matrix of claim 36, wherein the solid support is porous.

40. The matrix of claim 36, wherein the solid support is non-porous.

41. The matrix of claim 36, wherein the cap further comprises an aromatic hydrocarbon ring.

42. The matrix of claim 41, wherein at least one member of the aromatic hydrocarbon ring is the amine with a pK of less than about 9.

43. The matrix of claim 41, wherein the aromatic hydrocarbon ring is selected from the group consisting of pyridine and aniline.

44. The matrix of claim 36, wherein the second acidic moiety is a carboxylic acid residue.

45. The matrix of claim 36, wherein the spacer alkyl chain comprises two (2) to five (5) carbon atoms.

46. The matrix of claim 41, wherein the aromatic hydrocarbon covalently linked to the spacer define a basic amino acid moiety selected from the group consisting of histidine and histamine.

47. The matrix of claim 36, wherein the linker alkyl chain comprises three (3) to eight (8) carbon atoms.

48. The matrix of claim 36, wherein the linker alkyl chain includes at least one member selected from the group consisting of oxygen and amine.

49. The matrix of claim 30, wherein the linker is urea.

50. The matrix of claim 30, wherein the matrix is an anion exchanger capable of exchanging with the target nucleic acid at the first pH, neutral at a second pH which is higher than the first pH, and a cation exchanger at a third pH which is higher than the second pH.

51. The matrix of claim 44, wherein the second pH is at least about 4.0 and up to about pH 10.0.

52. The matrix of claim 30, wherein the proportion of the plurality of first ion exchange ligands and the plurality of second ion exchange ligands covalently attached to the solid phase is designed to ensure that when the matrix comes into contact with a solution comprising a target nucleic acid at the first pH, the matrix preferentially binds to the target nucleic acid.

53. The matrix of claim 30, wherein the matrix can be reused through at least two cycles of adherence of the target nucleic acid to the matrix at the first pH and release from the matrix at the desorption pH.

54. A method of isolating a target nucleic acid using a pH dependent ion exchange matrix, comprising the steps of:

(a) providing a pH dependent ion exchange matrix comprising:

a solid support, and

a plurality of first ion exchange ligands, each first ion exchange ligand comprising:

a cap comprising an amine with a pK of less than 9, wherein the amine is selected from the group consisting of a primary, a secondary, and a tertiary amine;

a spacer covalently attached to the cap, the spacer comprising a spacer alkyl chain with an amine terminus, and an acidic moiety covalently attached to the spacer alkyl chain; and

a linker comprising a linker alkyl chain covalently attached to the solid support at a first end of the linker alkyl chain and covalently attached to the amine terminus of the spacer at a second end of the linker alkyl chain;

wherein the matrix has a capacity to adsorb to a target nucleic acid at a first pH, and to release the target nucleic acid at a desorption pH which is higher than the first pH.

(b) provide a mixture comprising the target nucleic acid;

(c) combine the mixture and the matrix and incubate at the first pH until the nucleic acid adsorbs to the matrix, forming a complex;

(d) separate the complex from the mixture; and

(e) combine the complex with an elution solution at the desorption pH.

55. The method of claim 54, wherein the solid phase of the matrix provided in step (a) is a silica based material.

56. The method of claim 54, wherein the silica based material is glass fiber.

57. The method of claim 55, wherein the silica based material is a silica gel particle.

58. The method of claim 55, wherein the silica based material is a silica magnetic particle.

59. The method of claim 54, wherein the cap further comprises an aromatic hydrocarbon ring.

60. The method of claim 59, wherein the amine with a pK of less than about 9 is a member of the aromatic hydrocarbon ring.

61. The method of claim 54, wherein the spacer alkyl chain of the matrix provided in step (a) comprises two (2) to five (5) carbon atoms.

62. The method of claim 54, wherein the spacer of the matrix provided in step (a) is selected from the group consisting of cysteine and alanine.

63. The method of claim 54, wherein the aromatic hydrocarbon covalently linked to the spacer of the matrix provided in step (a) define a basic amino acid moiety selected from the group consisting of histidine and histamine.

64. The method of claim 54, wherein the linker alkyl chain of the matrix provided in step (a) comprises three (3) to eight (8) carbon atoms.

65. The method of claim 54, wherein the linker alkyl chain of the matrix provided in step (a) includes at least one member selected from the group consisting of oxygen, amine, and sulphur.

66. The method of claim 54, wherein the linker of the matrix provided in step (a) is selected from the group consisting of: glycidine and urea.

67. The method of claim 54, wherein the matrix provided in step (a) further comprises a plurality of second ion exchange ligands covalently attached to the solid phase.

68. The matrix of claim 54, wherein at least one of the plurality of second ion exchange ligands is a propionate residue.

69. The method of claim 54, wherein the mixture comprising the target nucleic acid material is obtained by disrupting biological material containing the target nucleic acid.

70. The method of claim 54, wherein the target nucleic acid material is RNA.

71. The method of claim 54, wherein the target nucleic acid is DNA.

72. The method of claim 54, wherein the pluralilty of ligands of the matrix provided in step (a) is selected from the group consisting of: histamine via epoxide, histamine via epoxide, histidine via urea, histidine via sulfhydryl, pyridyl alanine, pyridyl cysteine.

73. The method of claim 71, wherein the target nucleic acid is plasmid DNA.

74. The method of claim 71, wherein the target nucleic acid is genomic DNA.

75. A method of making a pH dependent ion exchange matrix, comprising the steps of:

(a) providing a solid phase;

(b) providing a linker comprising an alkyl chain having a first end and a second end; (c) combining the silica based solid phase and the linker under conditions where a covalent bond is formed between the solid phase and the first end of the linker alkyl chain, thereby producing a linker-modified solid phase;

(d) providing an acidic aromatic amine comprising: an aromatic hydrocarbon ring, wherein at least one member of the ring is an amine; a spacer which is covalently attached to the aromatic hydrocarbon, wherein the spacer comprises a spacer alkyl chain with an amino terminus; and an acidic substituent which is covalently attached to the spacer alkyl chain; and

(e) combining the linker-modified solid phase with the acidic aromatic amine under conditions where a covalent bond is formed between the amino terminus of the spacer alkyl chain of the acidic aromatic amine and the second end of the linker.

76. The method of claim 75, wherein the solid phase provided in step (a) is a silica based material.

77. The method of claim 76, wherein the linker is covalently attached to the solid phase in step (c) through a silica residue, wherein the silica residue is covalently attached to a first subunit and a second subunit, wherein the first subunit is selected from the group consisting of: -OH, $-OCH_3$, $-OCH_2CH_3$, and the second subunit is defined by the formula $-(OSiR^1_2)_x-R^1$, wherein R^1 is the same group as the first subunit, and x is at least 0.

78. The method of claim 76, wherein the silica based material is glass fiber.

79. The method of claim 76, wherein the silica based material is a silica gel particle.

80. The method of claim 79, wherein the silica gel particle is paramagnetic.

81. The method of claim 79, wherein the silica gel particle is porous.

82. The method of claim 79, wherein the silica gel particle is non-porous.

83. The method of claim 75, wherein the spacer is selected from a group consisting of cysteine and alanine.

84. The method of claim 75, wherein the aromatic hydrocarbon ring has at least five members.

85. The method of claim 75, wherein the acidic aromatic amine is an amino acid selected from the group consisting of histamine and histidine.

86. A method of making a pH dependent ion exchange matrix, comprising the steps of:

(a) providing a solid support;

(b) providing a first ion exchange ligand comprising:

a cap comprising an amine with a pK of less than 9, wherein the amine is selected from the group consisting of a primary, a secondary, or a tertiary amine;

a spacer covalently attached to the cap, the spacer comprising a spacer alkyl chain and with an amine terminus, an acidic substituent which is covalently attached to the spacer alkyl chain; and

a linker comprising a linker alkyl chain having a first end and a second end, wherein the second end is covalently attached to the amine terminus of the spacer;

(c) combining the solid phase and the first ion exchange ligand under conditions where a covalent bond is formed between solid phase and the first end of the linker alkyl chain.

87. The method of claim 86, wherein the first ion exchange ligand is an imidazole silylurea.

88. The method of claim 87, wherein the acidic substituent of the first ion exchange ligand is a carboxyl residue protected by a methyl group, wherein the methyl group is removed from the carboxyl residue after step (c).

89. The method of claim 86, wherein the method further comprises a step of covalently attaching a second ion exchange ligand precurser to the solid support, wherein the second ion exchange precurser includes an ion exchange terminus blocked by a protecting group.

90. The matrix of claim 89, wherein the method further comprises a step of removing the protecting group from the second ion exchange precurser, forming a second ion exchange ligand.

91. The method of claim 90, wherein the second ion exchange ligand is a cation exchanger at an acidic pH.

92. The method of claim 90, wherein the second ion exchange ligand is negatively charged at an acidic pH.

93. The method of claim 90, wherein relative proportions of a plurality of the first ion exchange residue and a plurality of the second ion exchange residue covalently attached to the solid phase are designed to control the charge ratio on the solid support surface, thereby controlling the binding affinity (capacity remains more a property of the available particle surface) of the solid support to bind to the target nucleic acid material.

94. The method of claim 86, wherein the solid support material is a silica gel particle.

95. The method of claim 94, wherein the silica gel particle is paramagnetic.

96. The method of claim 86, wherein the spacer is selected from a group consisting of cysteine and alanine.

97. The method of claim 86, wherein the cap further comprises an aromatic hydrocarbon ring having at least five members.

98. The method of claim 86, wherein the acidic cap and spacer comprise an amino acid selected from the group consisting of histamine and histidine.

99. A method of making a bimodal pH dependent ion exchange matrix, comprising the steps of:

(a) providing a solid support;

(b) providing a first ion exchange ligand comprising:

a cap comprising an amine with a pK of less than about 9, wherein the amine is selected from the group consisting of a primary, a secondary, or a teritary amine;

a spacer covalently attached to the cap, the spacer comprising a spacer alkyl chain and with an amine terminus; and

a linker comprising a linker alkyl chain having a first end and a second end, wherein the second end is covalently attached to the amine terminus of the spacer;

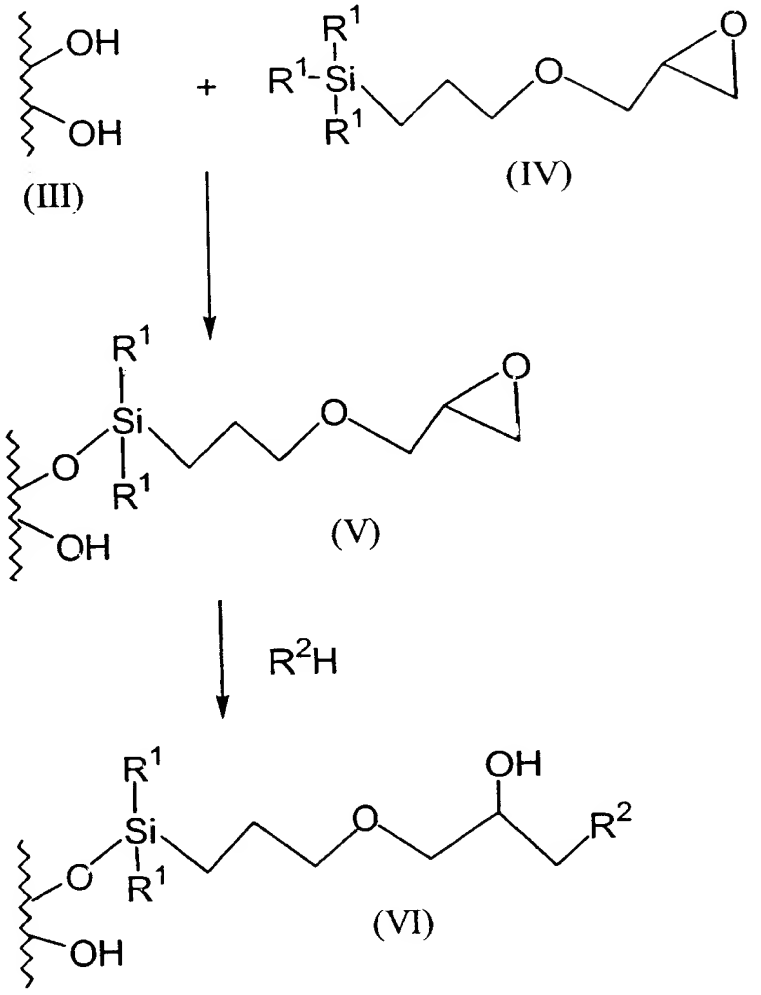
(c) combining the solid phase and the first ion exchange ligand under conditions where a covalent bond is formed between solid phase and the first end of the linker alkyl chain;

(d) providing a second ion exchange ligand, comprising a second alkyl chain and an acidic residue covalently attached thereto, wherein the acidic residue has a protective group covalently attached thereto;

(e) combining the solid phase with the first ion exchange ligand attached thereto with a second ligand under conditions which promote formation of a covalent bond between the protected second ion exchange ligand and the solid phase; and

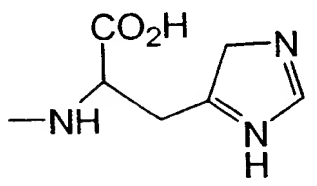
(f) deprotecting the acidic residue of the second anion exchange ligand by removing the protective group therefrom.

100. The method of claim 99, wherein the second ion exchange ligand is a propionate residue.



wherein, R^1 is -OH, $-OCH_3$, or $-OCH_2CH_3$; and

R^2 is:



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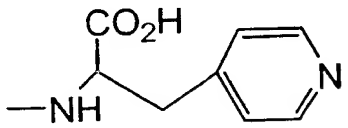

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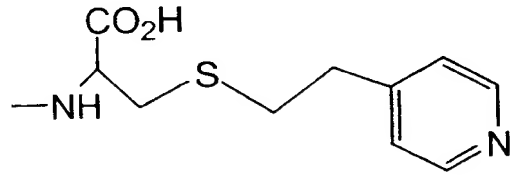
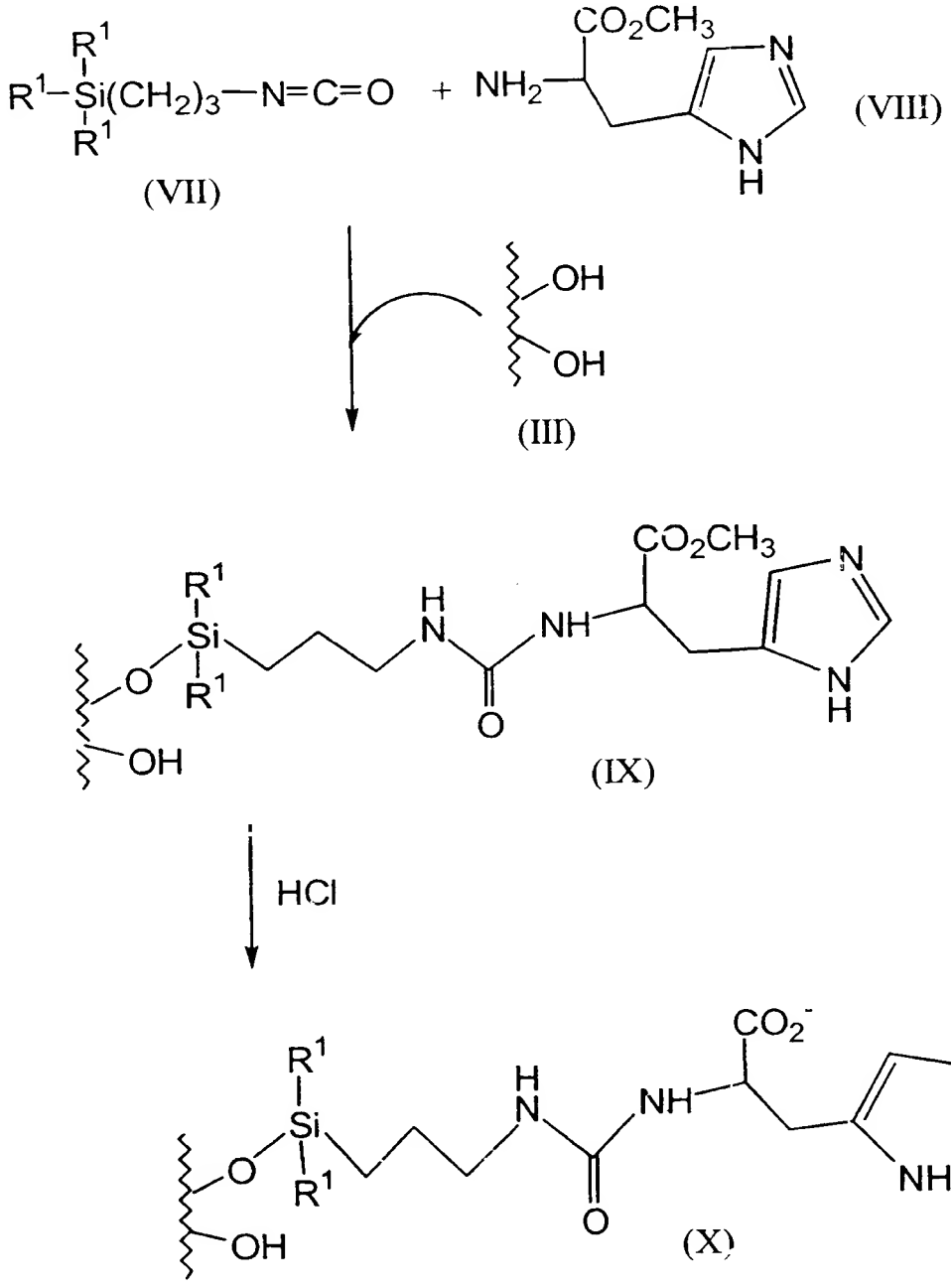
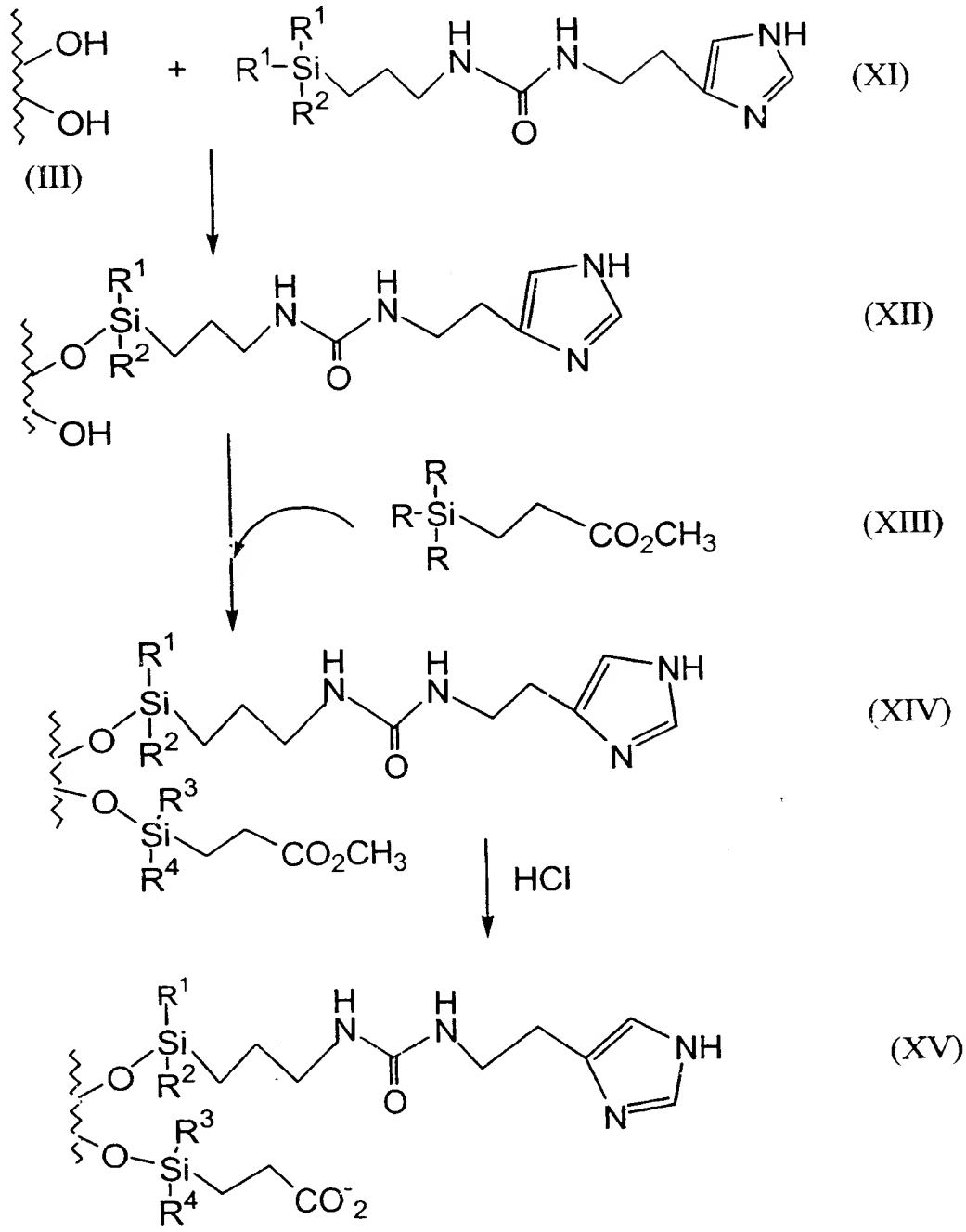


FIG. 1



wherein, R^1 is -OH, $-OCH_3$, or $-OCH_2CH_3$

FIG. 2



wherein, R^1 and R^3 are independently -OH, $-OCH_3$, or $-OCH_2CH_3$; R is -OH, $-OCH_3$, $-OCH_2CH_3$, or Cl; R^2 is $-(OSiR^1_2)_y-R^1$, wherein y is at least 0; and R^4 is $-(OSiR^3_2)_z-R^3$, wherein z is at least 0.

FIG. 3

FIG. 4

SEQUENCE LISTING

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<140> PCT/US00/_____
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<151> 1999-05-14

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<223> Oligonucleotide primer of the Adenomatous Polypoptosis Coli gene

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(71) Applicant: **PROMEGA CORPORATION** [US/US]; 2800 Woods Hollow Road, Madison, WI 53711-5399 (US).

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(74) Agents: **FRENCHICK, Grady, J.** et al.; Michael Best & Friedrich LLP, One South Pinckney Street, Suite 700, P.O. Box 1806, Madison, WI 53701-1806 (US).

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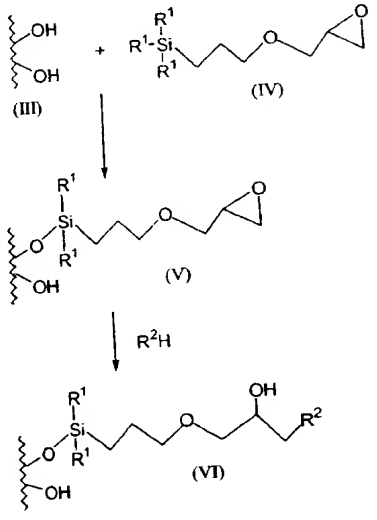
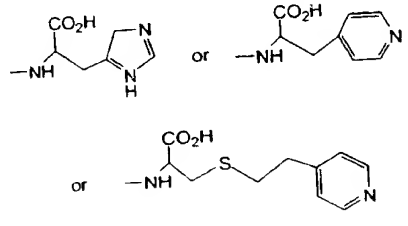
[Continued on next page]

(54) Title: pH DEPENDENT ION EXCHANGE MATRIX AND METHOD OF USE IN THE ISOLATION OF NUCLEIC ACIDS



wherein, R^1 is -OH, $-OCH_3$, or $-OCH_2CH_3$; and

R^2 is:



(57) Abstract: pH dependent ion exchange matrices are provided, with methods for making such matrices, and methods for using such matrices to isolate a target nucleic acid, as such as plasmid DNA, chromosomal DNA, or RNA from contaminants, including proteins, lipids, cellular debris, or other nucleic acids. Each pH dependent ion exchange matrix of this invention comprises at least two different ion exchange functional groups, one of which is capable of acting as an anion exchanger at a first pH, and the other of which is capable of acting as a cation exchanger at a second, higher pH. The matrix has an overall neutral charge in a pH range between the first and second pH. The pH dependent ion exchange matrices of the present invention are designed to bind to the target nucleic acid at a pH wherein the overall charge of the matrix is positive, and to release the target nucleic acid as the pH of the surrounding solution is increased. The target nucleic acid can be released from the pH dependent matrix in little or no salt and at about a neutral pH. The matrices and methods of this invention enable one to isolate a target nucleic acid in very few steps, without the use of hazardous chemicals. Target nucleic acids isolated using the pH dependent ion exchange matrices according to the present invention can be used immediately without further extraction or isolation.

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(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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Intern pal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 B01J41/06 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N B01J C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 652 348 A (HAGGARTY NEILL WARD ET AL) 29 July 1997 (1997-07-29) cited in the application figures 1-7 column 4, line 21 -column 6, line 14 ---	1,22,54, 75,86
A	US 5 898 071 A (HAWKINS TREVOR) 27 April 1999 (1999-04-27) abstract ---	22
A	L. W. MCLAUGHLIN: "Mixed-mode chromatography of nucleic acids" CHEM. REV., vol. 89, 1989, pages 309-319, XP002152137 page 312 -page 313 ---	36

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 2000	30/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	de Nooy, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/12186

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
F,X	BITNER R ET AL: "USE OF MAGNESILTM PARAMAGNETIC PARTICLES FOR PLASMID PURIFICATION, PCR CLEANUP, AND PURIFICATION OF DIDEOXY AND BIG DYE DNA SEQUENCING REACTIONS" PROCEEDINGS OF THE SPIE, 2000, XP000938886 the whole document	36-48,51

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern...al Application No
PCT/US 00/12186

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 3621295 A	09-04-1996
		CA 2200735 A	28-03-1996
		EP 0783366 A	16-07-1997
		JP 10506987 T	07-07-1998
		WO 9609116 A	28-03-1996
		NZ 293707 A	29-03-1999
		US 5945520 A	31-08-1999
US 5898071 A	27-04-1999	US 5705628 A	06-01-1998
		WO 9609379 A	28-03-1996

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A DOCPHOENIX

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Misc. Incoming Letter

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_____ ABST ____
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Affidavit or Exhibit Received

_____ APPENDIX ____
Appendix

_____ ARTIFACT ____
Artifact

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Bib Data Sheet

_____ CLM ____
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_____ COMPUTER ____
Computer Program Listing

_____ CRFL____
All CRF Papers for Backfile

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Terminal Disclaimer Filed

_____ DRW ____
Drawings

10/10/02 FOR 50
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Foreign Priority Papers

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IDS Including 1449

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Non-Patent Literature

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Oath or Declaration

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Petition

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Sequence Listing

_____ SPEC ____
Specification

_____ SPEC NO ____
Specification Not in English

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Transmittal New Application

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892

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Board of Appeals Decision

_____ APEA ____
Examiner Answer

_____ CTAV ____
Count Advisory Action

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Count Ex parte Quayle

_____ CTFR ____
Count Final Rejection

_____ CTNF ____
Count Non-Final

_____ CTRS ____
Count Restriction

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Examiner Interview

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DO/EO Acceptance

_____ M905 ____
DO/EO Missing Requirement

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Formal Drawing Required

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Petition Decision

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Change in Power of Attorney

_____ REM ____
Applicant Remarks in Amendment

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Extension of Time filed separate

Internal

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PTO Prepared Complete Claim Set

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Claim Worksheet

_____ WFEE ____
Fee Worksheet

File Wrapper

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File Wrapper Claim

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_____ SRFW ____
File Wrapper Search Info

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International Bureau

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(30) Priority Data:
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(71) Applicant: PROMEGA CORPORATION [US/US]; 2800 Woods Hollow Road, Madison, WI 53711-5399 (US).

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(74) Agents: FRENCHICK, Grady, J. et al.; Michael Best & Friedrich LLP, One South Pinckney Street, Suite 700, Madison, WI 53703 (US).

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Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CELL CONCENTRATION AND LYSATE CLEARANCE USING PARAMAGNETIC PARTICLES

(57) Abstract

Methods are disclosed for using paramagnetic particles to concentrate or harvest cells. Methods are also disclosed for clearing a solution of disrupted biological material, such as a lysate of cells or a homogenate of mammalian tissue. Methods are also disclosed for using paramagnetic particles to isolate target nucleic acids, such as RNA or DNA, from a solution cleared of disrupted biological material using the same type or a different type of paramagnetic particle. Kits are also disclosed for use with the various methods of the present invention. Nucleic acids isolated according to the present methods and using the present kits are suitable for immediate use in downstream processing, without further purification.

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CELL CONCENTRATION AND LYSATE CLEARANCE USING PARAMAGNETIC PARTICLES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Number 60/134,156, filed May 14, 1999.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

TECHNICAL FIELD

This invention relates generally to the use of magnetically responsive particles, such as magnetically responsive silica gel particles or magnetically responsive ion exchange particles, to harvest or to concentrate cells or biological tissue. This invention also relates to the use of such particles to clear lysates or homogenates of such cells or tissue. This invention relates, furthermore, to the use of such particles to isolate target nucleic acids, such as plasmid DNA, chromosomal DNA, DNA fragments, total RNA, mRNA, or RNA/DNA hybrids from non-target material in a cell lysate.

BACKGROUND OF THE INVENTION

Cells in a liquid culture must be concentrated or harvested before they can be preserved for later use, stained for direct analysis, or processed to isolate target specific materials therefrom. Most cell harvesting and concentration techniques involve centrifugation, filtration, or a combination of centrifugation and filtration. (See, e.g. Molecular Cloning, (1989) ed. by Sambrook et al., pp 2.22 and filtration system reference). Unfortunately, neither filtration nor centrifugation is amenable to automation. Specifically, neither can be performed at basic pipettor-diluter robotics stations, such as the Biomec®. When it becomes necessary to isolate or analyze certain types of material in the interior of a cell, such as a target nucleic acid or a protein, the cell membrane must be disrupted and the contents of the cell released into the solution surrounding the cell. Such disruption can be accomplished by mechanical means (e.g., by sonication or by blending in a mixer), by

enzymatic digestion (e.g. by digestion with proteases), or by chemical means (e.g., by alkaline lysis followed by addition of a neutralization solution). Whatever means is used to disrupt a cell, the end product, referred to herein as a lysate solution, consists of the target material and many contaminants, including cell debris. The lysate solution must be cleared of as many of the large contaminants as possible before the target material can be further isolated therefrom. Either or both of the same two means described above, i.e. centrifugation and filtration, have been used to clear lysate solutions prior to further processing. However, for reasons given above, neither means of clearing a lysate solution is amenable to automation.

Many different systems of materials and methods have been developed for use in the isolation of nucleic acids from cleared lysate solutions. Many such systems are silica based, such as those which employ controlled pore glass, filters embedded with silica particles, silica gel particles, resins comprising silica in the form of diatomaceous earth, glass fibers or mixtures of the above. Each such silica-based solid phase separation system is configured to reversibly bind nucleic acid materials when placed in contact with a medium containing such materials in the presence of chaotropic agents. The silica-based solid phases are designed to remain bound to the nucleic acid material while the solid phase is exposed to an external force such as centrifugation or vacuum filtration to separate the matrix and nucleic acid material bound thereto from the remaining media components. The nucleic acid material is then eluted from the solid phase by exposing the solid phase to an elution solution, such as water or an elution buffer. Numerous commercial sources offer silica-based resins designed for use in centrifugation and/or filtration isolation systems, e.g. Wizard® DNA purification systems products from Promega Corporation (Madison, Wisconsin, U.S.A.), or the QiaPrep® DNA isolation systems from Qiagen Corp. (Chatsworth, California, U.S.A.). Unfortunately, the type of silica-based solid phases described above all require one use centrifugation or filtration to perform the various isolation steps in each method, limiting the utility of such solid phases in automated systems.

Magnetically responsive solid phases, such as paramagnetic or superparamagnetic particles, offer an advantage not offered by any of the silica-based solid phases described above. Such particles could be separated from a solution by turning on and off a magnetic force field, or by moving a container on to and off of a magnetic separator. Such activities would be readily adaptable to automation.

Magnetically responsive particles have been developed for use in the isolation of nucleic acids. Such particles generally fall into either of two categories, those designed to reversibly bind nucleic acid materials directly, and those designed to reversibly bind nucleic acid materials through an intermediary. For an example of particles of the first type, see silica based porous particles designed to reversibly bind directly to DNA, such as MagneSil™ particles from Promega, or BioMag® magnetic particles from PerSeptive Biosystems. For examples of particles and systems of the second type designed to reversibly bind one particular type of nucleic acid (mRNA), see the PolyATract® Series 9600™ mRNA Isolation System from Promega Corporation (Madison, Wisconsin, U.S.A.); or the streptavidin coated microsphere particles from Bangs Laboratories (Carmel, Indiana, U.S.A.). Both of these systems employ magnetically responsive particles with streptavidin subunits covalently attached thereto, and biotin with an oligo(dT) moiety covalently attached thereto. The biotin-oligo(dT) molecules act as intermediaries, hybridizing to the poly(A) tail of mRNA molecules when placed into contact therewith, then binding to the streptavidin on the particles. The mRNA molecules are then released in water.

Indirect binding magnetic separation systems for nucleic acid isolation or separation require at least three components, i.e. magnetic particles, an intermediary, and a medium containing the nucleic acid material of interest. The intermediary/nucleic acid hybridization reaction and intermediary/particle binding reaction often require different solution and/or temperature reaction conditions from one another. Each additional component or solution used in the nucleic acid isolation procedure adds to the risk of contamination of the isolated end product by nucleases, metals, and other deleterious substances.

Various types of magnetically responsive silica based particles have been developed for use as solid phases in direct or indirect nucleic acid binding isolation methods. One such particle type is a magnetically responsive glass bead, preferably of a controlled pore size. See, e.g. Magnetic Porous Glass (MPG) particles from CPG, Inc. (Lincoln Park, New Jersey, U.S.A.); or porous magnetic glass particles described in U.S. Pat. No.'s 4,395,271; 4,233,169; or 4,297,337. Nucleic acid material tends to bind very tightly to glass, however, so that it can be difficult to remove once bound thereto. Therefore, elution efficiencies from magnetic glass particles tend to be low compared to elution efficiencies from particles containing lower amounts of a nucleic acid binding material such as silica.

Another type of magnetically responsive particle designed for use as a solid phase in direct binding and isolation of nucleic acids, particularly DNA, is a particle comprised of agarose embedded with smaller ferromagnetic particles and coated with glass, e.g. U.S. Patent 5,395,498. Yet another type of magnetically responsive particle designed for direct binding and isolation of nucleic acids is produced by incorporating magnetic materials into the matrix of polymeric silicon dioxide compounds, e.g. German Patent Application No. DE 43 07 262. The latter two types of magnetic particles, the agarose particle and the polymeric silicon dioxide matrix, tend to leach iron into a medium under the conditions required to bind nucleic acid materials directly to each such magnetic particle. It is also difficult to produce such particles with a sufficiently uniform and concentrated magnetic capacity to ensure rapid and efficient isolation of nucleic acid materials bound thereto.

Magnetically responsive beads designed for use in the isolation of target polymers, such as nucleic acids, and methods for their use therein are described in U.S. Pat. No. 5,681,946 and in International Publication No. WO 91/12079. These last beads are designed to become nonspecifically associated with the target polymer, only after the target polymer is precipitated out of a solution comprising the target polymer and the beads. Magnetic force is used to isolate the beads and polymer associated therewith from the solution. The magnetically responsive beads recommended for use in this last system are "finely divided magnetizable material encapsulated in organic polymer." ('946 Patent, col. 2, line 53).

A variety of solid phases have also been developed with ion exchange ligands capable of exchanging with nucleic acids. However, such systems are generally designed for use as a solid phase of a liquid chromatography system, for use in a filtration system, or for use with centrifugation to separate the solid phase from various solutions. Such systems range in complexity from a single species of ligand covalently attached to the surface of a filter, as in DEAE modified filters (e.g., CONCERT® isolation system, Life Technology Inc., Gaithersburg, MD, U.S.A.), to a column containing two different solid phases separated by a porous divider (e.g., U.S. Patent No. 5,660,984), to a chromatography resin with pH dependent ionizable ligands covalently attached thereto (e.g., U. S. Pat. No. 5,652,348).

Materials and methods are needed which enable one to automate as many steps as possible to quickly and efficiently isolate target nucleic acids from cells or mammalian tissue. Specifically, methods and materials are needed for the concentration or harvesting of cells, for the clearing of solutions of disrupted cells or tissue, and for the isolation of target